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14TH EDITION

Harrison's

PRINCIPLES of INTERNAL MEDICINE

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antibiotics, but it should be noted that all ulcers will become colonized with bacteria, and the purpose of antibiotic therapy should not be to clear all bacterial growth. Some ulcers may take months to heal or require skin grafting.

SEBORRHEIC DERMATITIS Seborrheic dermatitis is a common, chronic disorder characterized by greasy scales overlying erythematous patches or plaques. The most common location is in the scalp, where it may be recognized as severe dandruff. On the face, seborrheic dermatitis affects the eyebrows, eyelids, glabella, nasolabial fold, or ears. Scaling within the external ear is often mistaken for a chronic fungal infection (otomycosis), and postauricular dermatitis often becomes macerated and tender. Additionally, seborrheic dermatitis may develop in the central chest, axilla, groin, submammary folds, and gluteal cleft. Rarely, it may cause a widespread generalized dermatitis. Seborrheic dermatitis is usually symptomatic, with patients complaining of itching or burning.

Seborrheic dermatitis may be evident within the first few weeks of life, and within this context it occurs in the scalp ("cradle cap"), face, or groin. It is rarely seen in children beyond infancy but becomes evident again during adult life. Although it is frequently seen in patients with Parkinson's disease, in those who have had cerebrovascular accidents, and in those with human immunodeficiency virus (HIV) infection, the overwhelming majority of individuals with seborrheic dermatitis have no underlying disorder.

℞ TREATMENT

Treatment with low-potency topical glucocorticoids in conjunction with shampoos containing coal tar and/or salicylic acid is generally sufficient to control activity of this disorder. High potency topical glucocorticoid solutions (betamethasone or fluocinonide) are effective for control of scalp involvement. Fluorinated topical glucocorticoids should not be used on the face since this is often associated with the development of rebound worsening and steroid induced rosacea or atrophy.

PAPULOSQUAMOUS DISORDERS (Table 55-2)

PSORIASIS Psoriasis is one of the most common dermatologic diseases, affecting up to 1 to 2 percent of the world's population. It is a chronic inflammatory skin disorder clinically characterized by erythematous, sharply demarcated papules and rounded plaques, covered by silvery micaceous scale. The skin lesions of psoriasis are variably pruritic. Traumatized areas often develop lesions of psoriasis (Koebner or isomorphic phenomenon). Additionally, other external factors may exacerbate psoriasis including infections, stress, and medications (lithium, beta blockers, and antimalarials).

The most common variety of psoriasis is called *plaque type*. Patients with plaque-type psoriasis will have stable, slowly growing plaques, which remain basically unchanged for long periods of time. The most common areas for plaque psoriasis to occur are the elbows, knees, gluteal cleft, and the scalp. Involvement tends to be symmetrical. *Inverse psoriasis* affects the intertriginous regions including the axilla, groin, submammary region, and navel, and it also tends to affect the scalp, palms, and soles. The individual lesions are sharply demarcated plaques but may be moist due to their location. Plaque-type psoriasis generally develops slowly and runs an indolent course. It rarely spontaneously remits.

Eruptive psoriasis (guttate psoriasis) is most common in children and young adults. It develops acutely in individuals without psoriasis or in those with chronic plaque psoriasis. Patients present with many small eythematous, scaling papules, frequently after upper respiratory tract infection with beta-hemolytic streptococci. The differential diagnosis should include pityriasis rosea and secondary syphilis. Patients with psoriasis may also develop pustular lesions. These may be localized to the palms and soles or may be generalized and associated with fever, malaise, diarrhea, and arthralgias.

About half of all patients with psoriasis have fingernail involvement, appearing as punctate pitting, nail thickening, or subungual hyperkeratosis. About 5 to 10 percent of patients with psoriasis have associated joint complaints, and these are most often found in patients with fingernail involvement. Although some have the coincident occurrence of classic rheumatoid arthritis (see Chap. 313), many have joint disease that falls into one of five types associated with psoriasis: (1) disease limited to a single or a few small joints (70 percent of cases); (2) a seronegative rheumatoid arthritis–like disease; (3) involvement of the distal interphalangeal joints; (4) severe destructive arthritis with the development of "arthritis mutilans"; and (5) disease limited to the spine.

The etiology of psoriasis is still poorly understood, but there is clearly a genetic component. Over 50 percent of patients with psoriasis report a positive family history, and twin studies report a 65 to 72 percent concordance among monozygotic twins. Evidence has accumulated clearly indicating a role for T cells in the pathophysiology of psoriasis. Psoriasis may become particularly severe in individuals who are HIV-infected (see Chap. 308). Stimulation of immune function with cytokines such as interleukin 2 has been associated with abrupt worsening of preexisting psoriasis, and bone marrow transplantation has resulted in clearance of disease. In addition, agents that inhibit activated T cell function are often effective for the treatment of severe psoriasis.

℞ TREATMENT

Treatment of psoriasis depends on the type, location, and extent of disease. All patients should be instructed to avoid excess drying or irritation of their skin and to maintain adequate cutaneous hydration.

Table 55-2

Papulosquamous Disorders

	Clinical Features	Other Notable Features	Histologic Features
Psoriasis	Sharply demarcated, erythematous plaques with mica-like scale; predominantly elbows, knees, and scalp; atypical forms may localize to intertriginous areas; eruptive forms may be associated with infection (Reiter's syndrome)	May be aggravated by certain drugs, infection; severe forms seen associated with HIV	Acanthosis, vascular proliferation
Lichen planus	Purple polygonal papules marked by severe pruritus; lacy white markings, especially associated with mucous membrane lesions	Certain drugs may induce: thiazides, antimalarial drugs	Interface dermatitis
Pityriasis rosea	Rash often preceded by herald patch; oval to round plaques with trailing scale; most often affects the trunk, and eruption lines up in skin folds give a "fir tree"-like appearance; generally spares palms and soles	Variable pruritus; self-limited resolving in 2–8 weeks; may be imitated by secondary syphilis	Pathologic features often nonspecific
Dermatophytosis	Polymorphous appearance depending on dermatophyte, body site, and host response; sharply defined to ill-demarcated scaly plaques with or without inflammation; may be associated with hair loss	KOH preparation may show branching hyphae; culture helpful	Hyphae and neutrophils in stratum corneum

of Law will be entered on the same date herewith.

ORDER AND JUDGMENT

In accordance with the Findings of Fact and Conclusions of Law entered on the same date herewith,

IT IS HEREBY ORDERED AND ADJUDGED, as follows:

1. The Nolan patent (No. 4,506,189), issued on March 19, 1985, is a valid patent.

2. By the manufacture, production, sale and distribution of its SAF-T-COTE fluorescent lamp, Trojan has infringed the Nolan patent.

3. By virtue of this infringement, Shat-R-Shield is entitled to injunctive relief. Trojan shall immediately cease and desist from the manufacture, production, sale and distribution of the SAF-T-COTE fluorescent lamp.

4. Trojan shall recall all the SAF-T-COTE fluorescent lamps sold to and still in the possession of its customers.

5. The Court having determined that Trojan's infringement was not willful and wanton, Shat-R-Shield is not entitled to treble damages.

6. Shat-R-Shield shall have no accounting for monetary damages.

7. The Court having found that this is not an exceptional case, Shat-R-Shield is not entitled to its attorney's fees.

8. All claims having been resolved as to all parties herein, this action is now DISMISSED and STRICKEN from the docket.

9. There being no just reason for delay, this is a FINAL and APPEALABLE Order and Judgment.

Court of Appeals, Federal Circuit

In re Wands

No. 87-1454

Decided September 30, 1988

PATENTS

1. Patentability/Validity — Adequacy of disclosure (§115.12)

Data disclosed in application for immunoassay method patent, which shows that applicants screened nine of 143 cell lines developed for production of antibody necessary to practice invention, stored remainder of said cell lines, and found that four out of nine cell lines screened produced antibody falling within limitation of claims, were erroneously interpreted by Board of Patent Appeals and Interferences as failing to meet disclosure requirements of 35 USC 112, since board's characterization of stored cell lines as "failures" demonstrating unreliability of applicants' methods was improper in view of fact that such unscreened cell lines prove nothing concerning probability of success of person skilled in art attempting to obtain requisite antibodies using applicants' methods.

2. Patentability/Validity — Adequacy of disclosure (§115.12)

Disclosure in application for immunoassay method patent does not fail to meet enablement requirement of 35 USC 112 by requiring "undue experimentation," even though production of monoclonal antibodies necessary to practice invention first requires production and screening of numerous antibody producing cells or "hybridomas," since practitioners of art are prepared to screen negative hybridomas in order to find those that produce desired antibodies, since in monoclonal antibody art one "experiment" is not simply screening of one hybridoma but rather is entire attempt to make desired antibody, and since record indicates that amount of effort needed to obtain desired antibodies is not excessive, in view of applicants' success in each attempt to produce antibody that satisfied all claim limitations.

Appeal from decision of Patent and Trademark Office, Board of Patent Appeals and Interferences.

Application for patent of Jack R. Wands, Vincent R. Zurawski, Jr., and Hubert J. P. Schoemaker, serial number 188,735. From decision of Board of Patent Appeals and Interferences affirming rejection of application, applicants appeal. Reversed; Newman, J., concurring in part and dissenting in part in separate opinion.

Jorge A. Goldstein, of Saidman, Sterne, Kessler & Goldstein (Henry N. Wixon, with them on brief), Washington, D.C., for appellant.

John H. Raubitschek, associate solicitor (Joseph F. Nakamura and Fred E. McKelvey, with him on brief), PTO, for appellee.

Before Smith, Newman, and Bissell, circuit judges.

Smith, J.

This appeal is from the decision of the Patent and Trademark Office (PTO) Board of Patent Appeals and Interferences (board) affirming the rejection of all remaining claims in appellant's application for a patent, serial No. 188,735, entitled "Immunoassay Utilizing Monoclonal High Affinity IgM

Antibodies," which was filed September 19, 1980.[1] The rejection under 35 U.S.C. §112, first paragraph, is based on the grounds that appellant's written specification would not enable a person skilled in the art to make the monoclonal antibodies that are needed to practice the claimed invention without undue experimentation. We reverse.

I. *Issue*

The only issue on appeal is whether the board erred, as a matter of law, by sustaining the examiner's rejection for lack of enablement under 35 U.S.C. §112, first paragraph, of all remaining claims in appellants' patent application, serial No. 188,735.

II. *Background*

A. *The Art.*

The claimed invention involves immunoassay methods for the detection of hepatitis B surface antigen by using high-affinity monoclonal antibodies of the IgM isotype. *Antibodies* are a class of proteins (immunoglobulins) that help defend the body against invaders such as viruses and bacteria. An antibody has the potential to bind tightly to another molecule, which molecule is called an antigen. The body has the ability to make millions of different antibodies that bind to different antigens. However, it is only after exposure of an antigen that a complicated *immune response* leads to the production of antibodies against that antigen. For example, on the surface of hepatitis B virus particles there is a large protein called *hepatitis B surface antigen* (HBsAg). As its name implies, it is capable of serving as an antigen. During a hepatitis B infection (or when purified HBsAg is injected experimentally), the body begins to make antibodies that bind tightly and specifically to HBsAg. Such antibodies can be used as reagents for sensitive diagnostic tests (*e.g.*, to detect hepatitis B virus in blood and other tissues, a purpose of the claimed invention). A method for detecting or measuring antigens by using antibodies as reagents is called an *immunoassay*.

Normally, many different antibodies are produced against each antigen. One reason for this diversity is that different antibodies are produced that bind to different regions (determinants) of a large antigen molecule such as HBsAg. In addition, different antibodies may be produced that bind to the same determinant. These usually differ in the tightness with which they bind to the determinant. *Affinity* is a quantitative measure of the strength of antibody-antigen binding. Usually an antibody with a higher affinity for an antigen will be more useful for immunological diagnostic tests than one with a lower affinity. Another source of heterogeneity is that there are several immunoglobulin classes or *isotypes*. Immunoglobulin G (IgG) is the most common isotype in serum. Another isotype, immunoglobulin M (IgM), is prominent early in the immune response. IgM molecules are larger than IgG molecules, and have 10 antigen-binding sites instead of the 2 that are present in IgG. Most immunoassay methods use IgG, but the claimed invention uses only IgM antibodies.

For commercial applications there are many disadvantages to using antibodies from serum. Serum contains a complex mixture of antibodies against the antigen of interest within a much larger pool of antibodies directed at other antigens. There are available only in a limited supply that ends when the donor dies. The goal of monoclonal antibody technology is to produce an unlimited supply of a single purified antibody.

The blood cells that make antibodies are *lymphocytes*. Each lymphocyte makes only one kind of antibody. During an immune response, lymphocytes exposed to their particular antigen divide and mature. Each produces a *clone* of identical daughter cells, all of which secrete the same antibody. Clones of lymphocytes, all derived from a single lymphocyte, could provide a source of a single homogeneous antibody. However, lymphocytes do not survive for long outside of the body in cell culture.

Hybridoma technology provides a way to obtain large numbers of cells that all produce the same antibody. This method takes advantage of the properties of *myeloma* cells derived from a tumor of the immune system. The cancerous myeloma cells can divide indefinitely in vitro. They also have the potential ability to secrete antibodies. By appropriate experimental manipulations, a myeloma cell can be made to fuse with a lymphocyte to produce a single hybrid cell (hence, a hybridoma) that contains the genetic material of both cells. The hybridoma secretes the same antibody that was made by its parent lymphocyte, but acquires the capability of the myeloma cell to divide and grow indefinitely in cell culture. Antibodies produced by a clone of hybridoma cells (i.e., by hybridoma

[1] *In re Wands*, Appeal No. 673-76 (Bd. Pat. App. & Int. Dec. 30, 1986).

cells that are all progeny of a single cell) are called monoclonal antibodies.[2]

B. *The Claimed Invention.*

The claimed invention involves methods for the immunoassay of HBsAg by using high-affinity monoclonal IgM antibodies. Jack R. Wands and Vincent R. Zurawski, Jr., two of the three coinventors of the present application, disclosed methods for producing monoclonal antibodies against HBsAg in United States patent No. 4,271,145 (the '145 patent), entitled "Process for Producing Antibodies to Hepatitis Virus and Cell Lines Therefor," which patent issued on June 2, 1981. The '145 patent is incorporated by reference into the application on appeal. The specification of the '145 patent teaches a procedure for immunizing mice against HBsAg, and the use of lymphocytes from these mice to produce hybridomas that secrete monoclonal antibodies specific for HBsAg. The '145 patent discloses that this procedure yields both IgG and IgM antibodies with high-affinity binding to HBsAg. For the stated purpose of complying with the best mode requirement of 35 U.S.C. §112, first paragraph, a hybridoma cell line that secretes IgM antibodies against HBsAg (the 1F8 cell line) was deposited at the American Type Culture Collection, a recognized cell depository, and became available to the public when the '145 patent issued.

The application on appeal claims methods for immunoassay of HBsAg using monoclonal antibodies such as those described in the '145 patent. Most immunoassay methods have used monoclonal antibodies of the IgG isotype. IgM antibodies were disfavored in the prior art because of their sensitivity to reducing agents and their tendency to self-aggregate and precipitate. Appellants found that their monoclonal IgM antibodies could be used for immunoassay of HbsAg with unexpectedly high sensitivity and specificity. Claims 1, 3, 7, 8, 14, and 15 are drawn to methods for the immunoassay of HBsAg using high-affinity IgM monoclonal antibodies. Claims 19 and 25–27 are for chemically modified (*e.g.*, radioactively labeled) monoclonal IgM antibodies used in the assays. The broadest method claim reads:

1. An immunoassay method utilizing an antibody to assay for a substance comprising hepatitis B-surface antigen (HBsAg) determinants which comprises the steps of:

contacting a test sample containing said substance comprising HBsAg determinants with said antibody; and

determining the presence of said substance in said sample;

wherein said antibody is a monoclonal high affinity IgM antibody having a binding affinity constant for said HBsAg determinants of at least 10^9 M^{-1}.

Certain claims were rejected under 35 U.S.C. §103; these rejections have not been appealed. Remaining claims 1, 3, 7, 8, 14, 15, 19, and 25–27 were rejected under 35 U.S.C. §112, first paragraph, on the grounds that the disclosure would not enable a person skilled in the art to make and use the invention without undue experimentation. The rejection is directed solely to whether the specification enables one skilled in the art to make the monoclonal antibodies that are needed to practice the invention. The position of the PTO is that data presented by Wands show that the production of high-affinity IgM anti-HBsAg antibodies is unpredictable and unreliable, so that it would require undue experimentation for one skilled in the art to make the antibodies.

III. *Analysis*

A. *Enablement by Deposit of Micro-organisms and Cell Lines.*

The first paragraph of 35 U.S.C. §112 requires that the specification of a patent must enable a person skilled in the art to make and use the claimed invention. "Patents * * * are written to enable those skilled in the art to practice the invention."[3] A patent need not disclose what is well known in the art.[4] Although we review underlying facts found by the board under a "clearly erroneous" standard,[5] we review enablement as a question of law.[6]

Where an invention depends on the use of living materials such as microorganisms or

[2] For a concise description of monoclonal antibodies and their use in immunoassay see *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1368–71, 231 USPQ 81, 82–83 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987).

[3] *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984).

[4] *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

[5] *Coleman v. Dines*, 754 F.2d 353, 356, 224 USPQ 857, 859 (Fed. Cir. 1985).

[6] *Moleculon Research Corp. v. CBS, Inc.*, 793 F.2d 1261, 1268, 229 USPQ 805, 810 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 875 (1987); *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960 n.6, 220 USPQ 592, 599 n.6 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 835 [225 USPQ 232] (1984).

cultured cells, it may be impossible to enable the public to make the invention (i.e., to obtain these living materials) solely by means of a written disclosure. One means that has been developed for complying with the enablement requirement is to deposit the living materials in cell depositories which will distribute samples to the public who wish to practice the invention after the patent issues.[7] Administrative guidelines and judicial decisions have clarified the conditions under which a deposit of organisms can satisfy the requirements of section 112.[8] A deposit has been held necessary for enablement where the starting materials (i.e., the living cells used to practice the invention, or cells from which the required cells can be produced) are not readily available to the public.[9] Even when starting materials are available, a deposit has been necessary where it would require undue experimentation to make the cells of the invention from the starting materials.[10]

In addition to satisfying the enablement requirement, deposit of organisms also can be used to establish the filing date of the application as the prima facie date of invention,[11] and to satisfy the requirement under 35 U.S.C. §114 that the PTO be guaranteed access to the invention during pendency of the application.[12] Although a deposit may serve these purposes, we recognized, in *In re Lundak*,[13] that these purposes, nevertheless, may be met in ways other than by making a deposit.

A deposit also may satisfy the best mode requirement of section 112, first paragraph, and it is for this reason that the 1F8 hybridoma was deposited in connection with the '145 patent and the current application. Wands does not challenge the statements by the examiner to the effect that, although the deposited 1F8 line enables the public to perform immunoassays with antibodies produced by that single hybridoma, the deposit does not enable the generic claims that are on appeal. The examiner rejected the claims on the grounds that the written disclosure was not enabling and that the deposit was inadequate. Since we hold that the written disclosure fully enables the claimed invention, we need not reach the question of the adequacy of deposits.

B. *Undue Experimentation.*

Although inventions involving microorganisms or other living cells often can be enabled by a deposit,[14] a deposit is not always necessary to satisfy the enablement requirement.[15] No deposit is necessary if the biological organisms can be obtained from readily available sources or derived from readily available starting materials through routine screening that does not require undue experimentation.[16] Whether the specification in an application involving living cells (here, hybridomas) is enabled without a deposit must be decided on the facts of the particular case.[17]

Appellants contend that their written specification fully enables the practice of

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[7] *In re Argoudelis*, 434 F.2d 1390, 1392–93, 168 USPQ 99, 101–02 (CCPA 1970).

[8] *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (Fed. Cir. 1985); *Feldman v. Aunstrup*, 517 F.2d 1351, 186 USPQ 108 (CCPA 1975), *cert. denied*, 424 U.S. 912 [188 USPQ 720] (1976); Manual of Patent Examining Procedure (MPEP) 608.01 (p)(C) (5th ed. 1983, rev. 1987). *See generally* Hampar, *Patenting of Recombinant DNA Technology: The Deposit Requirement*, 67 J. Pat. Trademark Off. Soc'y 569 (1985).

[9] *In re Jackson*, 217 USPQ 804, 807–08 (Bd. App. 1982) (strains of a newly discovered species of bacteria isolated from nature); *Feldman*, 517 F.2d 1351, 186 USPQ 108 (uncommon fungus isolated from nature); *In re Argoudelis*, 434 F.2d at 1392, 168 USPQ at 102 (novel strain of antibiotic-producing microorganism isolated from nature); *In re Kropp*, 143 USPQ 148, 152 (Bd. App. 1959) (newly discovered microorganism isolated from soil).

[10] *Ex parte Forman*, 230 USPQ 546, 547 (Bd. Pat. App. & Int. 1986) (genetically engineered bacteria where the specification provided insufficient information about the amount of time and effort required); *In re Lundak*, 773 F.2d 1216, 227 USPQ 90 (unique cell line produced from another cell line by mutagenesis).

[11] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96; *In re Feldman*, 517 F.2d at 1355, 186 USPQ at 113; *In re Argoudelis*, 434 F.2d at 1394–96, 168 USPQ at 103–04 (Baldwin, J. concurring).

[12] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96; *In re Feldman*, 517 F.2d at 1354, 186 USPQ at 112.

[13] *In re Lundak*, 773 F.2d at 1222, 227 USPQ at 95–96.

[14] *In re Argoudelis*, 434 F.2d at 1393, 168 USPQ at 102.

[15] *Tabuchi v. Nubel*, 559 F.2d 1183, 194 USPQ 521 (CCPA 1977).

[16] *Id.* at 1186–87, 194 USPQ at 525; *Merck & Co. v. Chase Chem. Co.*, 273 F.Supp. 68, 77, 155 USPQ 139, 146 (D.N.J. 1967); *Guaranty Trust Co. v. Union Solvents Corp.*, 54 F.2d 400, 403–06, 12 USPQ 47, 50–53 (D. Del. 1931), *aff'd*, 61 F.2d 1041, 15 USPQ 237 (3d Cir. 1932), *cert. denied*, 288 U.S. 614 (1933); MPEP 608.01(p)(C) ("No problem exists when the microorganisms used are known and readily available to the public.").

[17] *In re Jackson*, 217 USPQ at 807; *see In re Metcalfe*, 410 F.2d 1378, 1382, 161 USPQ 789, 792 (CCPA 1969).

their claimed invention because the monoclonal antibodies needed to perform the immunoassays can be made from readily available starting materials using methods that are well known in the monoclonal antibody art. Wands states that application of these methods to make high-affinity IgM anti-HBsAg antibodies requires only routine screening, and that does not amount to undue experimentation. There is no challenge to their contention that the starting materials (i.e., mice, HBsAg antigen, and myeloma cells) are available to the public. The PTO concedes that the methods used to prepare hybridomas and to screen them for high-affinity IgM antibodies against HBsAg were either well known in the monoclonal antibody art or adequately disclosed in the '145 patent and in the current application. This is consistent with this court's recognition with respect to another patent application that methods for obtaining and screening monoclonal antibodies were well known in 1980.[18] The sole issue is whether, in this particular case, it would require undue experimentation to produce high-affinity IgM monoclonal antibodies.

Enablement is not precluded by the necessity for some experimentation such as routine screening.[19] However, experimentation needed to practice the invention must not be undue experimentation.[20] "the key word is 'undue,' not 'experimentation.' "[21]

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. Ansul Co. v. Uniroyal, Inc. [448 F.2d 872, 878-79; 169 USPQ 759, 762-63 (2d Cir. 1971), *cert. denied*, 404 U.S. 1018 [172 USPQ 257] (1972)]. The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed * * *.[22]

The term "undue experimentation" does not appear in the statute, but it is well established that enablement requires that the specification teach those in the art to make and use the invention without undue experimentation.[23] Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations. The board concluded that undue experimentation would be needed to practice the invention on the basis of experimental data presented by Wands. These data are not in dispute. However, Wands and the board disagree strongly on the conclusion that should be drawn from that data.

Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in *Ex parte Forman*.[24] They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.[25]

In order to understand whether the rejection was proper, it is necessary to discuss further the methods for making specific monoclonal antibodies. The first step for making monoclonal antibodies is to immunize an animal. The '145 patent provides a detailed description of procedures for immunizing a specific strain of mice against HBsAg. Next the spleen, an organ rich in lymphocytes, is removed and the lymphocytes are separated from the other spleen cells. The lymphocytes are mixed with myeloma cells, and the mixture is treated to cause a few of the cells to fuse with each other. Hybridoma cells that secrete the desired antibodies then must be isolated from the enormous number of other cells in the mixture. This is done through a series of screening procedures.

The first step is to separate the hybridoma cells from unfused lymphocytes and myeloma cells. The cells are cultured in a medi-

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[18] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94.

[19] *Id.*; *Atlas Powder Co. v. E.I. DuPont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir. 1984); *In re Angstadt*, 537 F.2d at 502-504, 190 USPQ at 218; *In re Geerdes*, 491 F.2d 1260, 1265, 180 USPQ 789, 793 (CCPA 1974); *Mineral Separation, Ltd. v. Hyde*, 242 U.S. 261, 270-71 (1916).

[20] *Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *W.L. Gore*, 721 F.2d at 1557, 220 USPQ at 316; *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977) (Miller, J., concurring).

[21] *In re Angstadt*, 537 F.2d at 504, 190 USPQ at 219.

[22] *In re Jackson*, 217 USPQ at 807.

[23] *See Hybritech*, 802 F.2d at 1384, 231 USPQ at 94; *Atlas Powder*, 750 F.2d at 1576, 224 USPQ at 413.

[24] *Ex parte Forman*, 230 USPQ at 547.

[25] *Id.*; *see In re Colianni*, 561 F.2d at 224, 195 USPQ at 153 (Miller, J., concurring); *In re Rainer*, 347 F.2d 574, 577, 146 USPQ 218, 221 (CCPA 1965).

um in which all the lymphocytes and myeloma cells die, and only the hybridoma cells survive. The next step is to isolate and clone hybridomas that make antibodies that bind to the antigen of interest. Single hybridoma cells are placed in separate chambers and are allowed to grow and divide. After there are enough cells in the clone to produce sufficient quantities of antibody to analyze, the antibody is assayed to determine whether it binds to the antigen. Generally, antibodies from many clones do not bind the antigen, and these clones are discarded. However, by screening enough clones (often hundreds at a time), hybridomas may be found that secrete antibodies against the antigen of interest.

Wands used a commercially available radioimmunoassay kit to screen clones for cells that produce antibodies directed against HBsAg. In this assay the amount of radioactivity bound gives some indication of the strength of the antibody-antigen binding, but does not yield a numerical affinity constant, which must be measured using the more laborious Scatchard analysis. In order to determine which anti-HBsAg antibodies satisfy all of the limitations of appellants' claims, the antibodies require further screening to select those which have an IgM isotype and have a binding affinity constant of at least 10^9 M^{-1}.[26] The PTO does not question that the screening techniques used by Wands were well known in the monoclonal antibody art.

During prosecution Wands submitted a declaration under 37 C.F.R §1.132 providing information about all of the hybridomas that appellants had produced before filing the patent application. The first four fusions were unsuccessful and produced no hybridomas. The next six fusion experiments all produced hybridomas that made antibodies specific for HBsAg. Antibodies that bound at least 10,000 cpm in the commercial radioimmunoassay were classified as "high binders." Using this criterion, 143 high-binding hybridomas were obtained. In the declaration, Wands stated that [27]

It is generally accepted in the art that, among those antibodies which are binders with 50,000 cpm or higher, there is a very high likelihood that high affinity (Ka [greater than] 10^9 M^{-1}) antibodies will be found. However, high affinity antibodies can also be found among high binders of between 10,000 and 50,000, as is clearly demonstrated in the Table.

The PTO has not challenged this statement.

The declaration stated that a few of the high-binding monoclonal antibodies from two fusions were chosen for further screening. The remainder of the antibodies and the hybridomas that produced them were saved by freezing. Only nine antibodies were subjected to further analysis. Four (three from one fusion and one from another fusion) fell within the claims, that is, were IgM antibodies and had a binding affinity constant of at least 10^9 M^{-1}. Of the remaining five antibodies, three were found to be IgG, while the other two were IgM for which the affinity constants were not measured (although both showed binding well above 50,000 cpm).

Apparently none of the frozen cell lines received any further analysis. The declaration explains that after useful high-affinity IgM monoclonal antibodies to HBsAg had been found, it was considered unnecessary to return to the stored antibodies to screen for more IgMs. Wands says that the existence of the stored hybridomas was disclosed to the PTO to comply with the requirement under 37 C.F.R. §1.56 that applicants fully disclose all of their relevant data, and not just favorable results.[28] How these stored hybridomas are viewed is central to the positions of the parties.

The position of the board emphasizes the fact that since the stored cell lines were not completely tested, there is no proof that any of them are IgM antibodies with a binding affinity constant of at least 10^9 M^{-1}. Thus, only 4 out of 143 hybridomas, or 2.8 percent, were *proved* to fall within the claims. Furthermore, antibodies that were proved to be high-affinity IgM came from only 2 of 10 fusion experiments. These statistics are viewed by the board as evidence that appellants' methods were not predictable or reproducible. The board concludes that Wands' low rate of demonstrated success shows that a person skilled in the art would have to

[26] The examiner, the board, and Wands all point out that, technically, the strength of antibody-HBsAg binding is measured as *avidity*, which takes into account multiple determinants on the HBsAg molecule, rather than affinity. Nevertheless, despite this correction, all parties then continued to use the term "affinity." We will use the terminology of the parties. Following the usage of the parties, we will also use the term "high-affinity" as essentially synonymous with "having a binding affinity constant of at least 10^9 M^{-1}."

[27] A table in the declaration presented the binding data for antibodies from every cell line. Values ranged from 13,867 to 125,204 cpm, and a substantial proportion of the antibodies showed binding greater than 50,000 cpm. In confirmation of Dr. Wand's statement, two antibodies with binding less than 25,000 cpm were found to have affinity constants greater than 10^9 M^{-1}.

[28] *See Rohm & Haas Co. v. Crystal Chem. Co.*, 722 F.2d 1556, 220 USQ 98 (Fed. Cir. 1983).

engage in undue experimentation in order to make antibodies that fall within the claims.

Wands views the data quite differently. Only nine hybridomas were actually analyzed beyond the initial screening for HBsAg binding. Of these, four produced antibodies that fell within the claims, a respectable 44 percent rate of success. (Furthermore, since the two additional IgM antibodies for which the affinity constants were never measured showed binding in excess of 50,000 cpm, it is likely that these also fall within the claims.) Wands argues that the remaining 134 unanalyzed, stored cell lines should not be written off as failures. Instead, if anything, they represent partial success. Each of the stored hybridomas had been shown to produce a high-binding antibody specific for HBsAg. Many of these antibodies showed binding above 50,000 cpm and are thus highly likely to have a binding affinity constant of at least 10^9 M^{-1}. Extrapolating from the nine hybridomas that were screened for isotype (and from what is well known in the monoclonal antibody art about isotype frequency), it is reasonable to assume that the stored cells include some that produce IgM. Thus, if the 134 incompletely analyzed cell lines are considered at all, they provide some support (albeit without rigorous proof) to the view that hybridomas falling within the claims are not so rare that undue experimentation would be needed to make them.

The first four fusion attempts were failures, while high-binding antibodies were produced in the next six fusions. Appellants contend that the initial failures occurred because they had not yet learned to fuse cells successfully. Once they became skilled in the art, they invariably obtained numerous hybridomas that made high-binding antibodies against HBsAg and, in each fusion where they determined isotype and binding affinity they obtained hybridomas that fell within the claims.

Wands also submitted a second declaration under 37 C.F.R. §1.132 stating that after the patent application was submitted they performed an eleventh fusion experiment and obtained another hybridoma that made a high-affinity IgM anti-HBsAg antibody. No information was provided about the number of clones screened in that experiment. The board determined that, because there was no indication as to the number of hybridomas screened, this declaration had very little value. While we agree that it would have been preferable if Wands had included this information, the declaration does show that when appellants repeated their procedures they again obtained a hybridoma that produced an antibody that fit all of the limitations of their claims.

[1] We conclude that the board's interpretation of the data is erroneous. It is strained and unduly harsh to classify the stored cell lines (each of which was proved to make high-binding antibodies against HBsAg) as failures demonstrating that Wands' methods are unpredictable or unreliable.[29] At worst, they prove nothing at all about the probability of success, and merely show that appellants were prudent in not discarding cells that might someday prove useful. At best, they show that high-binding antibodies, the starting materials for IgM screening and Scatchard analysis, can be produced in large numbers. The PTO's position leads to the absurd conclusion that the more hybridomas an applicant makes and saves without testing the less predictable the applicant's results become. Furthermore, Wands' explanation that the first four attempts at cell fusion failed only because they had not yet learned to perform fusions properly is reasonable in view of the fact that the next six fusions were all successful. The record indicates that cell fusion is a technique that is well known to those of ordinary skill in the monoclonal antibody art, and there has been no claim that the fusion step should be more difficult or unreliable where the antigen is HBsAg than it would be for other antigens.

[2] When Wands' data is interpreted in a reasonable manner, analysis considering the factors enumerated in *Ex parte Forman* leads to the conclusion that undue experimentation would not be required to practice the invention. Wands' disclosure provides considerable direction and guidance on how to practice their invention and presents working examples. There was a high level of skill in the art at the time when the application was filed, and all of the methods needed to practice the invention were well known.

The nature of monoclonal antibody technology is that it involves screening hybridomas to determine which ones secrete antibody with desired characteristics. Practitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody. No evidence was presented by either party on how many hybridomas would be viewed by those in the art as requiring undue experimentation to screen. However, it seems unlikely that un-

[29] Even if we were to accept the PTO's 2.8% success rate, we would not be required to reach a conclusion of undue experimentation. Such a determination must be made in view of the circumstances of each case and cannot be made solely by reference to a particular numerical cutoff.

due experimentation would be defined in terms of the number of hybridomas that were never screened. Furthermore, in the monoclonal antibody art it appears that an "experiment" is not simply the screening of a single hybridoma, but is rather the entire attempt to make a monoclonal antibody against a particular antigen. This process entails immunizing animals, fusing lymphocytes from the immunized animals with myeloma cells to make hybridomas, cloning the hybridomas, and screening the antibodies produced by the hybridomas for the desired characteristics. Wands carried out this entire procedure three times, and was successful each time in making at least one antibody that satisfied all of the claim limitations. Reasonably interpreted, Wands' record indicates that, in the production of high-affinity IgM antibodies against HBsAG, the amount of effort needed to obtain such antibodies is not excessive. Wands' evidence thus effectively rebuts the examiner's challenge to the enablement of their disclosure.[30]

IV. *Conclusion*

Considering all of the factors, we conclude that it would not require undue experimentation to obtain antibodies needed to practice the claimed invention. Accordingly, the rejection of Wands' claims for lack of enablement under 35 U.S.C. §112, first paragraph, is reversed.

REVERSED

Newman, J., concurring in part, dissenting in part.

A

I concur in the court's holding that additional samples of hybridoma cell lines that produce these high-affinity IgM monoclonal antibodies need not be deposited. This invention, as described by Wands, is not a selection of a few rare cells from many possible cells. To the contrary, Wands states that all monoclonally produced IgM antibodies to hepatitis B surface antigen have the desired high avidity and other favorable properties, and that all are readily preparable by now-standard techniques.

Wands states that his United States Patent No. 4,271,145 describes fully operable techniques, and is distinguished from his first four failed experiments that are referred to in the Rule 132 affidavit. Wands argues that these biotechnological mechanisms are relatively well understood and that the preparations can be routinely duplicated by those of skill in this art, as in *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1380, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 107 S.Ct. 1606 (1987). I agree that it is not necessary that there be a deposit of multiple exemplars of a cell system that is readily reproduced by known, specifically identified techniques.

B

I would affirm the board's holding that Wands has not complied with 35 U.S.C. §112, first paragraph, in that he has not provided data sufficient to support the breadth of his generic claims. Wands' claims on appeal include the following:

> 19. Monoclonal high affinity IgM antibodies immunoreactive with HBsAg determinants, wherein said antibodies are coupled to an insoluble solid phase, and wherein the binding affinity constant of said antibodies for said HBsAg determinants is at least 10^9 M^{-1}.
>
> 26. Monoclonal high affinity IgM antibodies immunoreactive with hepatitis B surface antigen.

Wands states that he obtained 143 "high binding monoclonal antibodies of the right specificity" in the successful fusions; although he does not state how they were determined to be high binding or of the right specificity, for Wands also states that only nine of these 143 were tested.

Of these nine, four (three from one fusion and one from another fusion) were found to have the claimed high affinity and to be of the IgM isotype. Wands states that the other five were either of a different isotype or their affinities were not determined. (This latter statement also appears to contradict his statement that all 143 were "high binding".)

Wands argues that a "success rate of four out of nine", or 44.4%, is sufficient to support claims to the entire class. The Commissioner deems the success rate to be four out of 143, or 2.8%; to which Wands responds with statistical analysis as to how unlikely it is that Wands selected the only four out of 143 that worked. Wands did not, however, prove the right point. The question is whether Wands, by testing nine out of 143 (the Commissioner points out that the randomness of the sample was not established), and finding that four out of the nine had the desired properties, has provided sufficient experimental support for the breadth of the requested claims, in the context that "experi-

[30] *In re Strahilevitz*, 668 F.2d 1229, 1232, 212 USPQ 561, 563 (CCPA 1982).

ments in genetic engineering produce, at best, unpredictable results", quoting from *Ex parte Forman*, 230 USPQ 546, 547 (Bd.Pat.App. and Int. 1986).

The premise of the patent system is that an inventor, having taught the world something it didn't know, is encouraged to make the product available for public and commercial benefit, by governmental grant of the right to exclude others from practice of that which the inventor has disclosed. The boundary defining the excludable subject matter must be carefully set: it must protect the inventor, so that commercial development is encouraged; but the claims must be commensurate with the inventor's contribution. Thus the specification and claims must meet the requirements of 35 U.S.C. §112. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 23-24 (CCPA 1970).

As the science of biotechnology matures the need for special accommodation, such as the deposit of cell lines or microorganisms, may diminish; but there remains the body of law and practice on the need for sufficient disclosure, including experimental data when appropriate, that reasonably support the scope of the requested claims. That law relates to the sufficiency of the description of the claimed invention, and if not satisfied by deposit, must independently meet the requirements of Section 112.

Wands is not claiming a particular, specified IgM antibody. He is claiming all such monoclonal antibodies in assay for hepatitis B surface antigen, based on his teaching that such antibodies have uniformly reproducible high avidity, free of the known disadvantages of IgM antibodies such as tendency to precipitate or aggregate. It is incumbent upon Wands to provide reasonable support for the proposed breadth of his claims. I agree with the Commissioner that four exemplars shown to have the desired properties, out of the 143, do not provide adequate support.

Wands argues that the law should not be "harsher" where routine experiments take a long time. However, what Wands is requesting is that the law be less harsh. As illustrated in extensive precedent on the question of how much experimentation is "undue", each case must be determined on its own facts. *See, e.g., W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1557, 220 USPQ 303, 316 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 218 (CCPA 1976); *In re Cook*, 439 F.2d 730, 734-35, 169 USPQ 298, 302-03 (CCPA 1971).

The various criteria to be considered in determining whether undue experimentation is required are discussed in, for example, *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1971); *In re Rainer*, 347 F.2d 574, 146 USPQ 218 (CCPA 1965); *Ex parte Forman*, 230 USPQ at 547. Wands must provide sufficient data or authority to show that his results are reasonably predictable within the scope of the claimed generic invention, based on experiment and/or scientific theory. In my view he has not met this burden.

Patent and Trademark Office
Trademark Trial and Appeal Board

In re Johanna Farms Inc.

Serial No. 542,343

Decided June 30, 1988

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Prior adjudication — In general (§410.1501)

Trademark Trial and Appeal Board's prior decision upholding examiner's refusal to register proposed mark "La Yogurt" does not preclude registration of mark pursuant to subsequent application, since applicant, by presenting survey evidence and consumer letters regarding issue of how purchasers perceive proposed mark, has demonstrated that instant factual situation is different from situation presented in prior proceeding.

TRADEMARKS AND UNFAIR TRADE PRACTICES

2. Types of marks — Non-descriptive — Particular marks (§327.0505)

Term "La Yogurt," with "yogurt" disclaimed, is registrable, since word "yogurt" is common English generic term rather than corruption or misspelling of French word for yogurt, since examining attorney failed to meet burden of showing clear evidence of generic use of mark as whole, and since evidence of record, including survey and consumer letters to applicant, demonstrates that primary significance of "La Yogurt" to majority of relevant public is that of brand name rather than generic term.

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TREATMENT OF RHEUMATOID ARTHRITIS WITH CHIMERIC MONOCLONAL ANTIBODIES TO TUMOR NECROSIS FACTOR α

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Objective. To evaluate the safety and efficacy of a chimeric monoclonal antibody to tumor necrosis factor α (TNFα) in the treatment of patients with rheumatoid arthritis (RA).

Methods. Twenty patients with active RA were treated with 20 mg/kg of anti-TNFα in an open phase I/II trial lasting 8 weeks.

Results. The treatment was well tolerated, with no serious adverse events. Significant improvements were seen in the Ritchie Articular Index, which fell from a median of 28 at study entry to a median of 6 by week 6 ($P < 0.001$), the swollen joint count, which fell from 18 to 5 ($P < 0.001$) over the same period, and in the other major clinical assessments. Serum C-reactive protein levels fell from a median of 39.5 mg/liter at study entry to 8 mg/liter at week 6 ($P < 0.001$), and significant decreases were also seen in serum amyloid A and interleukin-6 levels.

Conclusion. Treatment with anti-TNFα was safe and well tolerated and resulted in significant clinical and laboratory improvements. These preliminary results support the hypothesis that TNFα is an important regulator in RA, and suggest that it may be a useful new therapeutic target in this disease.

Despite optimal use of current antirheumatic therapy, the outcome for many patients with rheumatoid arthritis (RA) consists of pain, disability, and premature death (1–3). As a response to the need for more effective and less toxic treatment, and to an increase in our understanding of the pathogenic mechanisms in RA, several groups have used monoclonal antibodies as therapeutic agents in this disease (4–10). Such immunotherapy has been, in most cases, targeted specifically to the T cell, a strategy based on evidence that T cells are involved in the initiation and maintenance of RA (11).

Here, we outline an alternative immunotherapeutic strategy, which involves the use of monoclonal antibodies with specificity for a cytokine, tumor necrosis factor α (TNFα). This approach is based on a body of knowledge regarding the role of cytokines in general, and of TNFα in particular, in the inflammatory process in RA. The first clearly documented study demonstrated the presence of interleukin-1 (IL-1) in RA synovial fluid (12). Subsequently, we and others have reported the presence and local synthesis in

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Table 1. Demographic features of 20 patients with refractory rheumatoid arthritis

Patient	Age/sex	Disease duration (years)	Previous DMARDs*	Concomitant therapy†
1	48/F	7	SSZ, DP, GST, AUR, MTX, AZA, HCQ	Pred. 5 mg
2	63/F	7	SSZ, GST, DP	Para. 1–2 gm
3	59/M	3	AUR, HCQ, GST, MTX, SSZ	Pred. 10 mg, Indo. 225 mg
4	56/M	10	GST, DP, AZA, SSZ	Pred. 12.5 mg, Ibu. 2 gm, Para. 1–2 gm
5	28/F	3	GST, SSZ, DP, AZA	Pred. 8 mg, Para. 1–2 gm, Code. 16 mg
6	40/M	3	SSZ, HCQ, AUR	Nap. 1 gm
7	54/F	7	DP, GST, SSZ, AZA, MTX	Para. 1–2 gm, Code. 16–32 mg
8	23/F	11	HCQ, GST, SSZ, MTX, AZA	Pred. 7.5 mg, Dicl. 100 mg, Para. 1–2 gm, Dext. 100–200 mg
9	51/F	15	GST, HCQ, DP, MTX	Pred. 7.5 mg, Dicl. 125 mg, Para. 1–3 gm
10	47/F	12	SSZ, CYC, MTX	Ben. 4 gm
11	34/F	10	DP, SSZ, MTX	Pred. 10 mg, Para. 1.5 gm, Code. 30–90 mg
12	57/F	12	GST, MTX, DP, AUR	Asp. 1.2 gm
13	51/F	7	SSZ, AZA	Para. 1–4 gm
14	72/M	11	GST, DP, AZA, MTX	Pred. 5 mg, Para. 1–4 gm, Code. 16–64 mg
15	51/F	17	HCQ, DP, SSZ, MTX	Asp. 0.3 gm
16	62/F	16	GST, DP, SSZ, MTX, AZA	Para. 1–4 gm, Code. 16–64 mg
17	56/F	11	SSZ, DP, GST, MTX, HCQ, AZA	Pred. 7.5 mg, Eto. 600 mg, Para. 1–2 gm, Dext. 100–200 mg
18	48/F	14	GST, MTX, DP, SSZ, AUR, AZA	Pred. 7.5 mg, Indo. 100 mg, Para. 1–3 gm
19	42/F	3	SSZ, MTX	Fen. 450 mg, Ben. 6 gm, Code. 30 mg
20	47/M	20	GST, DP, SSZ, AZA	Pred. 10 mg, Para. 1–3 gm

* Disease-modifying antirheumatic drugs (DMARDs) were SSZ = sulfasalazine; DP = D-penicillamine; GST = gold sodium thiomalate; AUR = auranofin; MTX = methotrexate; AZA = azathioprine; HCQ = hydroxychloroquine; CYC = cyclophosphamide.
† Daily doses are shown. Pred. = prednisolone; Para. = paracetamol; Indo. = indomethacin; Ibu. = ibuprofen; Code. = codeine phosphate; Nap. = naprosyn; Dicl. = diclofenac; Dext. = dextropropoxyphene; Ben. = benorylate; Asp. = aspirin; Eto. = etodolac; Fen. = fenbufen.

rheumatoid synovial membrane of many cytokines, including IL-1 (13), TNFα (13,14), IL-6 (15), granulocyte-macrophage colony-stimulating factor (GM-CSF; 16), IL-8 (17), and transforming growth factor β (TGFβ) (18,19).

We have investigated the relationships between these cytokines in RA, using a synovial culture system in which dissociated rheumatoid synovial cells are allowed to spontaneously re-aggregate in vivo. Even in the absence of extrinsic stimulation, such cells express high levels of cytokines and HLA class II molecules (20). Using this system, we showed that production of bioactive IL-1 was abrogated by neutralizing antibodies to TNFα, but not by antibodies to TNFβ or by normal rabbit IgG (21). This occurred in rheumatoid, but not osteoarthritic, cultures and suggested to us that TNFα was of particular importance as a regulatory cytokine. Subsequent analysis reinforced this concept, with the demonstration that another proinflammatory cytokine, GM-CSF, was regulated in the synovial membrane by TNFα (22) and that TNFα receptor expression, necessary for transmitting TNFα signals, was up-regulated in rheumatoid synovium (23,24).

Two recent mouse studies provide further insight into the importance of TNFα in arthritis. Keffer et al (25) described a mouse transgenic for the human TNFα gene, which expressed high levels of human TNFα in vivo and which reproducibly developed arthritis beginning at 4 weeks of age. The disease in these animals could be prevented by administration of monoclonal antibodies to human TNFα. In separate experiments in our own laboratory, we showed that in the type II collagen arthritis model in the DBA/1 mouse, the hamster anti-murine TNF monoclonal antibody TN3.19.2 significantly ameliorated the inflammation and tissue destruction when administered before or after the onset of disease (26).

Based on these considerations, it was of interest to determine the effect of therapy with a chimeric (human IgG1, murine Fv) monoclonal antibody to human TNFα in patients with rheumatoid arthritis. We report here that anti-TNFα therapy was safe and well tolerated, and induced marked improvements in both clinical and laboratory disease measures. These findings are consistent with our postulate concerning the critical role of TNFα in the pathogenesis of RA (27,28), and suggest that TNFα may be a useful therapeutic target in this disease.

Table 2. Changes in clinical assessments following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Morning stiffness, minutes	Pain score, 0–10 cm	Ritchie index, 0–69	Swollen joint count, 0–28	Grip strength, 0–300 mm Hg		IDA, 1–4	Patient's assessment, no. grades improved, 0–3
					Left hand	Right hand		
Screen	135, 0–600	7.4, 4–9.7	23, 4–51	16, 4–28	84, 45–300	96, 57–300	3, 2.3–3.3	NA
0	180, 20–600	7.1, 2.7–9.7	28, 4–52	18, 3–27	77, 52–295	92, 50–293	3, 2–3.5	NA
1	20, 0–180 (<0.001†)	2.6, 0.6–7.8 (<0.001†)	13, 2–28 (<0.001; <0.002†)	13.5, 1–25 (>0.05)	122, 66–300 (>0.05)	133, 57–300 (>0.05)	2, 1.5–3.3 (<0.001†)	1, 1–3
2	15, 0–150 (<0.001†)	3.0, 0.3–6.4 (<0.001†)	13, 1–28 (<0.001†)	11.5, 1–22 (<0.003; <0.02†)	139, 75–300 (<0.03; >0.05†)	143, 59–300 (>0.05)	2, 1.5–3.2 (<0.001†)	1.5, 1–3
3	5, 0–150 (<0.001†)	2.2, 0.2–7.4 (<0.001†)	8, 0–22 (<0.001†)	6, 1–19 (<0.001; <0.002†)	113, 51–300 (>0.05)	142, 65–300 (>0.05)	2, 1.2–3.2 (<0.001†)	2, 1–2
4	15, 0–90 (<0.001†)	1.9, 0.1–5.6 (<0.001†)	10, 0–17 (<0.001†)	6, 0–21 (<0.001; <0.002†)	124, 79–300 (<0.02; >0.05†)	148, 64–300 (<0.03; >0.05†)	1.8, 1.3–2.7 (<0.001†)	2, 1–2
6	5, 0–90 (<0.001†)	1.9, 0.1–6.2 (<0.001†)	6, 0–18 (<0.001†)	5, 1–14 (<0.001†)	119, 68–300 (<0.04; >0.05†)	153, 62–300 (<0.05; >0.05†)	1.7, 1.3–2.8 (<0.001†)	2, 1–2
8	15, 0–60 (<0.001†)	2.1, 0.2–7.7 (<0.001†)	8, 1–28 (<0.001†)	7, 1–18 (<0.001†)	117, 69–300 (<0.03; >0.05†)	167, 53–300 (<0.03; >0.05†)	1.8, 1.5–2.8 (<0.001†)	2, 1–3

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. All *P* values versus week 0, by Mann-Whitney test. IDA = Index of Disease Activity; NA = not applicable.
† Adjusted for multiple statistical comparisons.

PATIENTS AND METHODS

Patient selection. Twenty patients were recruited, each of whom fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the diagnosis of RA (29). The clinical characteristics of the patients are shown in Table 1. The study group comprised 15 females and 5 males, with a median age of 51 years (range 23–72), a median disease duration of 10.5 years (range 3–20), and a history of failed therapy with standard disease-modifying antirheumatic drugs (DMARDs) (median number of failed DMARDs 4, range 2–7).

Seventeen patients were seropositive at study entry or had been seropositive at some stage of their disease. All had erosions evident on radiographs of the hands or feet, and 3 had rheumatoid nodules. All patients had active disease at trial entry, as defined by an Index of Disease Activity (IDA) (30) of at least 1.75, together with at least 3 swollen joints, and were classified in anatomic and functional stage II or III (31). The pooled data for each of the clinical and laboratory indices of disease activity at the time of screening for the trial (up to 4 weeks prior to trial entry), and on the day of trial entry itself (week 0), are shown in Tables 2 and 3.

All DMARDs were discontinued at least 1 month prior to trial entry. Patients were allowed to continue taking a nonsteroidal antiinflammatory drug and/or prednisolone (≤12.5 mg/day) during the trial. The dosage of these agents was kept stable for 1 month prior to trial entry and during the course of the trial. No parenteral corticosteroids were allowed during these periods. Simple analgesics were allowed ad libitum.

Patients with other serious medical conditions were excluded from study. Specific exclusions were as follows: serum creatinine >150 μmoles/liter (normal 60–120), hemoglobin (Hgb) <90 gm/liter (normal 120–160 in females, and 135–175 in males), white blood cell (WBC) count <4 × 10^9/liter (normal 4–11 × 10^9/liter), platelet count <100 × 10^9/liter (normal 150–400 × 10^9/liter), and abnormal liver enzyme levels or active pathology noted on chest radiographs.

All patients gave their informed consent for the trial, and approval was granted by the local ethics committee.

Treatment protocol. cA2 is a chimeric human/mouse monoclonal anti-TNFα antibody, consisting of the constant regions of human (Hu)IgG1κ, coupled to the Fv region of a high-affinity neutralizing murine anti-HuTNFα antibody (A2). The antibody was produced by Centocor Inc., by continuous fermentation of a mouse myeloma cell line which had been transfected with cloned DNA coding for cA2, and was purified from culture supernatant by a series of steps involving column chromatography. The chimeric antibody retains specificity for natural and recombinant HuTNFα, and is of high affinity.

The antibody was stored at 4°C in 20-ml vials containing 5 mg of cA2 per milliliter of 0.01*M* phosphate buffered saline in 0.15*M* sodium chloride at a pH of 7.2 and was filtered through a 0.2-μm sterile filter before use. The appropriate amount of cA2 was then diluted to a total volume of 300 ml in sterile saline and administered intravenously via a 0.2-μm in-line filter over a period of 2 hours.

Patients were admitted to the hospital for 8–24 hours for each treatment, and were mobile except during infusions. The trial was of an open, uncontrolled design, with a comparison of 2 treatment schedules. Patients 1–5 and 11–20 received a total of 2 infusions, each consisting of 10 mg/kg of cA2, at entry to the study (week 0) and 14 days later (week 2). Patients 6–10 received a total of 4 infusions of 5 mg/kg at cA2, at entry and on days 4, 8, and 12. The total dose received by the 2 patient groups was therefore the same: 20 mg/kg.

Assessments. *Safety monitoring.* Vital signs were recorded every 15–30 minutes during infusions, and at intervals for up to 24 hours postinfusion. Patients were

Table 3. Changes in laboratory measures following treatment of rheumatoid arthritis patients with cA2*

Week of trial	Hgb, gm/liter	WBC, $\times 10^9$/liter	Platelets, $\times 10^9$/liter	ESR, mm/hour	CRP, mg/liter	SAA, mg/ml	RF, inverse titer
Screen	117, 98–146	7.9, 3.9–15.2	352, 274–631	59, 18–87	42, 9–107	ND	ND
0	113, 97–144	9.0, 4.9–15.7	341, 228–710	55, 15–94	39.5, 5–107	245, 18–1,900	2,560, 160–10,240
1	114, 96–145 (>0.05)	8.5, 3.6–13.6 (>0.05)	351, 223–589 (>0.05)	26, 13–100 (>0.05)	5, 0–50 (<0.001†)	58, 0–330 (<0.001; <0.003†)	ND
2	112, 95–144 (>0.05)	8.2, 4.3–12.7 (>0.05)	296, 158–535 (<0.04; >0.05†)	27, 10–90 (<0.02; >0.05†)	5.5, 0–80 (<0.001; <0.003†)	80, 11–900 (<0.02; <0.04†)	ND
3	110, 89–151 (>0.05)	9.0, 3.7–14.4 (>0.05)	289, 190–546 (<0.03; >0.05†)	27, 12–86 (<0.04; >0.05†)	7, 0–78 (<0.001; <0.002†)	ND	ND
4	112, 91–148 (>0.05)	8.2, 4.7–13.9 (>0.05)	314, 186–565 (>0.05)	23, 10–87 (<0.04; >0.05†)	10, 0–91 (<0.004; <0.02†)	ND	ND
6	116, 91–159 (>0.05)	9.1, 2.9–13.9 (>0.05)	339, 207–589 (>0.05)	23, 12–78 (<0.03; >0.05†)	8, 0–59 (<0.001†)	ND	ND
8	114, 94–153 (>0.05)	7.6, 4.2–13.5 (>0.05)	339, 210–591 (>0.05)	30, 7–73 (>0.05)	6, 0–65 (<0.001†)	ND	480, 40–5,120 (>0.05)

* Values are the median, range (*P*) for 20 patients for the initial screen and weeks 0–2, and for 19 patients thereafter. Patient 15 dropped out after week 2 of study. For rheumatoid factor (RF), only those patients with week 0 titers ≥1:160 in the particle agglutination assay were included (n = 14). All *P* values versus week 0, by Mann-Whitney test. Normal ranges: hemoglobin (Hgb) 120–160 gm/liter in females and 135–175 gm/liter in males; white blood cell (WBC) count 4–11 × 10^9/liter; platelet count 150–400 × 10^9/liter; erythrocyte sedimentation rate (ESR) <15 mm/hour in females and <10 mm/hour in males; C-reactive protein (CRP) <10 mg/liter; serum amyloid A (SAA) <10 mg/ml. ND = not done.

questioned concerning possible adverse events before each infusion and at weeks 1, 2, 3, 4, 6, and 8 of the trial. A complete physical examination was performed at screening and at week 8. In addition, patients were monitored by standard laboratory tests including a complete blood cell count, and levels of C3 and C4 components of complement, IgG, IgM, and IgA, serum electrolytes, creatinine, urea, alkaline phosphatase, aspartate transaminase, and total bilirubin.

Sample times for these tests were between 0800 and 0900 hours (preinfusion) and 1200–1400 hours (24 hours postinfusion). Blood tests subsequent to day 1 were performed in the morning, usually between 0700 and 1200 hours. Urine analysis and culture were also performed at each assessment point.

Response assessment. The patients were assessed for response to cA2 at weeks 1, 2, 3, 4, 6, and 8 of the trial. The assessments were all made between 0700 and 1300 hours by the same observer (AL-F). The following clinical assessments were made: duration of morning stiffness (minutes), pain score (0–10 cm on a visual analog scale), Ritchie Articular Index (maximum score 69) (32), number of swollen joints (28 joint count) (validation described in ref. 33), grip strength (0–300 mm Hg, mean of 3 measurements per hand, by sphygmomanometer cuff), and an assessment of function (the Stanford Health Assessment Questionnaire [HAQ], modified for British patients [34]). In addition, the patients' global assessments of response were recorded using a 5-point scale (worse, no response, fair response, good response, excellent response).

Routine laboratory indicators of disease activity included complete blood cell counts, C-reactive protein (CRP) levels (by rate nephelometry), and the erythrocyte sedimentation rate (ESR; Westergren). Followup assessments were made at monthly intervals after the conclusion of the formal trial period, in order to assess the duration of response.

Analysis of improvement in individual patients was made using two separate indices. First, an IDA was calculated for each time point according to the method of Mallya and Mace (30), with input variables of morning stiffness, pain score, Ritchie Articular Index, grip strength, ESR, and Hgb. The second index calculated was that of Paulus et al (35), which uses input variables of morning stiffness, ESR, joint pain/tenderness, joint swelling, and patient's and physician's global assessments of disease severity.

To calculate the presence (or otherwise) of a response according to this index, two approximations were made to accommodate our data. The swollen joint count used by us (nongraded total of swollen joints of 28 joints assessed), which has been validated (33), was used in place of the more extensive graded count described by Paulus et al, and the patient's and physician's global assessments of *response* recorded by us were approximated to the global assessments of *disease activity* used by Paulus et al (35). In addition to determining response according to these published indices, we selected 6 disease activity assessments of interest (morning stiffness, pain score, Ritchie Articular Index, swollen joint count, ESR, and CRP) and calculated their mean percentage improvement. We have used this value to give an indication of the degree of improvement seen in responding patients.

Immunologic investigations. Rheumatoid factors were measured using the rheumatoid arthritis particle agglutination assay (RAPA) (FujiBerio Inc, Tokyo, Japan), in which titers of 1:160 or greater were considered significant. Rheumatoid factor isotypes were measured by enzyme-linked immunosorbent assay (ELISA) (Cambridge Life Sciences, Ely, UK). Addition of cA2, at concentrations of up to 200 μg/ml, to these assay systems did not alter the assay results (data not shown).

Antinuclear antibodies were detected by immunoflu-

orescence on HEp-2 cells (Biodiagnostics, Upton, UK), and antibodies to extractable nuclear antigens were measured by counterimmunoelectrophoresis with polyantigen extract (Biodiagnostics). Sera positive by immunofluorescence were also screened for antibodies to DNA by the Farr assay (Kodak Diagnostics, Amersham, UK). Anticardiolipin antibodies were measured by ELISA (Shield Diagnostics, Dundee, Scotland). Serum amyloid A (SAA) was measured by sandwich ELISA (Biosource International, Camarillo, CA). Antiglobulin responses to the infused chimeric antibody were measured by an in-house ELISA, using cA2 as a capture reagent.

Cytokine assays. Bioactive TNF was measured in sera using the WEHI 164 clone 13 cytotoxicity assay (36). Total IL-6 was measured in sera using a commercial immunoassay (Medgenix Diagnostics, Brussels, Belgium) and using a sandwich ELISA developed in-house, with monoclonal antibodies provided by Dr. F. di Padova (Basel, Switzerland). Microtiter plates were coated with monoclonal antibody LNI 314-14 at a concentration of 3 μg/ml for 18 hours at 4°C, and blocked with 3% bovine serum albumin in 0.1*M* phosphate buffered saline, pH 7.2. Undiluted sera or standards (recombinant HuIL-6, 0–8.1 μg/ml) were added to the wells in duplicate and incubated for 18 hours at 4°C. Bound IL-6 was detected by incubation with monoclonal antibody LNI 110-14 for 90 minutes at 37°C, followed by biotin-labeled goat anti-murine IgG2b for 90 minutes at 37°C (Southern Biotechnology, Birmingham, AL). The assay was developed using streptavidin–alkaline phosphatase (Southern Biotechnology) and *p*-nitrophenyl phosphate as a substrate, and the optical density read at 405 nm.

Statistical analysis. Data for week 0 versus subsequent time points were compared for each assessment using the Mann-Whitney test. For comparison of rheumatoid factor titers (by RAPA), the data were expressed as dilutions before applying the test.

This was an exploratory study, in which prejudgments about the optimal times for assessment were not possible. Although it has not been common practice to adjust for multiple statistical comparisons in such studies (4–10), a conservative statistical approach would require adjustment of *P* values to take into account analysis at several time points. The *P* values have therefore been presented in two forms: unadjusted, and after making allowance for analysis at multiple time points by use of the Bonferroni adjustment. Where *P* values remained <0.001 after adjustment, a single value only is given. A *P* value of <0.05 is considered significant.

RESULTS

Safety of cA2. The administration of cA2 was exceptionally well tolerated, with no headache, fever, hemodynamic disturbance, allergy, or other acute manifestation. No serious adverse events were recorded during the 8-week trial. Two minor infective episodes were recorded, each "possibly related" to cA2: patient 15 presented at week 2 with clinical features of bronchitis. Sputum culture grew only normal commensals. She had a history of smoking and of a similar illness 3 years previously. The illness responded promptly to treatment with amoxicillin, but her second cA2 infusion was withheld and the data for this patient are therefore not analyzed beyond week 2. Patient 18 showed significant bacteriuria on routine culture at week 6 ($>10^5$/ml; lactose-fermenting coliform), but was asymptomatic. This condition also responded promptly to amoxicillin.

Routine analysis of blood samples showed no consistent adverse changes in hematologic parameters, renal function, liver function, or levels of C3, C4, or immunoglobulins during the 8 weeks of the trial. Four minor, isolated, and potentially adverse laboratory disturbances were recorded. Patient 2 experienced a transient rise in blood urea levels, from 5.7 mmoles/liter to 9.2 mmoles/liter (normal 2.5–7), with no change in serum creatinine. This change was associated with the temporary use of a diuretic, which had been prescribed for a non-rheumatologic disorder. The value normalized within 1 week and was classified as "probably not related" to cA2.

Patient 6 experienced a transient fall in the peripheral blood lymphocyte count, from 1.6×10^9/liter to 0.8×10^9/liter (normal 1.0–4.8). This abnormality was not seen at the next sample point (2 weeks later), was not associated with any clinical manifestations, and was classified as "possibly related" to cA2. Patients 10 and 18 developed elevated titers of anti-DNA antibodies at weeks 6 and 8 of the trial. Elevated anticardiolipin antibodies were also detected in patient 10. Both patients had a preexisting positive antinuclear antibody titer, and patient 10 had a history of borderline lymphocytopenia and high serum IgM. There were no clinical features of systemic lupus erythematosus, and the laboratory changes were judged "probably related" to cA2.

Efficacy of cA2. The pattern of response for each of the clinical assessments of disease activity and the derived IDA are shown in Table 2. All clinical assessments showed improvement following treatment with cA2, with maximal responses from week 3. Duration of morning stiffness decreased from a median of 180 minutes at study entry (week 0) to 5 minutes at week 6 ($P < 0.001$ by Mann-Whitney test, adjusted), representing a 97% improvement. The pain score decreased from 7.1 to 1.9 over the same period ($P < 0.001$, adjusted), representing an improvement of 73%. Similarly, the Ritchie Articular Index improved from 28 to 6 at week 6 ($P < 0.001$, adjusted; 79% improvement), and the swollen joint count decreased from 18

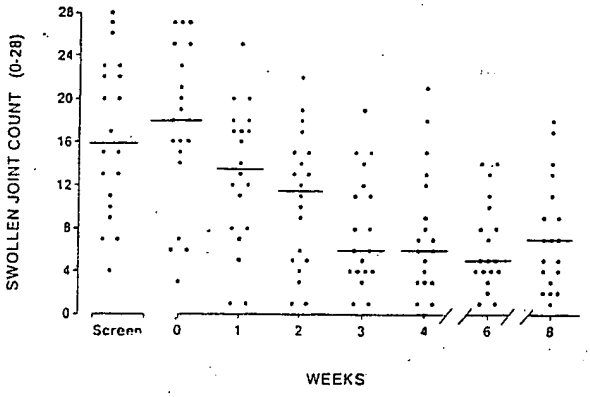

Figure 1. Swollen joint counts (maximum 28), as recorded by a single observer, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P > 0.05$ at week 1, $P < 0.02$ at week 2, $P < 0.002$ at weeks 3 and 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

to 5 ($P < 0.001$, adjusted; 72% improvement). The individual swollen joint counts for all time points are shown in Figure 1.

Grip strength also improved; the median grip strength rose from 77 mm Hg (left) and 92 mm Hg (right) at week 0 to 119 (left) and 153 (right) at week 6 ($P < 0.04$ and $P < 0.05$, left and right hands, respectively; $P > 0.05$ both hands, adjusted for multiple comparisons). The IDA has a range of 1 (normal) to 4 (severe disease activity). The IDA showed a decrease from a median of 3 at study entry to 1.7 at week 6 ($P < 0.001$, adjusted). Patients were asked to grade their responses to cA2 using a 5-point scale. No patient recorded a response of "worse" or "no change" at any point in the trial. "Fair," "good," and "excellent" responses were classified as improvements of 1, 2, and 3 grades, respectively. At week 6, there was a median of 2 grades of improvement (Table 2).

We also measured changes in the patients' functional capacity, using the HAQ, as modified for British patients (range 0–3). The median (range) HAQ score improved from 2 (0.9–3) at study entry to 1.1 (0–2.6) by week 6 ($P < 0.001$ and $P < 0.002$ adjusted).

The changes in the laboratory values which reflect disease activity are shown in Table 3. The most rapid and impressive changes were seen in serum CRP levels, which fell from a median of 39.5 mg/liter at week 0 (normal <10) to 8 mg/liter by week 6 of the trial ($P < 0.001$, adjusted), representing an improvement of 80%. Of the 19 patients with elevated CRP at study entry, 17 showed decreases to the normal range at some point during the trial. The improvement in CRP was maintained in most patients over the assessment period (Table 3 and Figure 2); the exceptions with high values at 4 and 6 weeks tended to be those with the highest starting values (data not shown).

The ESR also showed improvement, with a fall from 55 mm/hour at study entry (normal <10 in males and <15 in females) to 23 mm/hour at week 6 ($P < 0.03$ and $P > 0.05$ adjusted; 58% improvement). SAA levels were elevated in all patients at trial entry, and fell from a median of 245 mg/ml (normal <10) to 58 mg/ml at week 1 ($P < 0.003$ adjusted; 76% improvement) and to 80 mg/ml at week 2 ($P < 0.04$, adjusted). No significant changes were seen in Hgb level, WBC count, or platelet count at week 6, although the platelet count did improve at weeks 2 and 3 compared with trial entry (Table 3).

The response data were also analyzed for each patient individually (not shown). The majority of patients had their best overall responses at week 6, at which time 13 assessed their responses as "good" while 6 assessed their responses as "fair." Eighteen of the 19 patients who completed the treatment schedule achieved an improvement in the IDA of 0.5 or greater at week 6, and 10 achieved an improvement of 1.0 or greater. All patients achieved a response at week 6

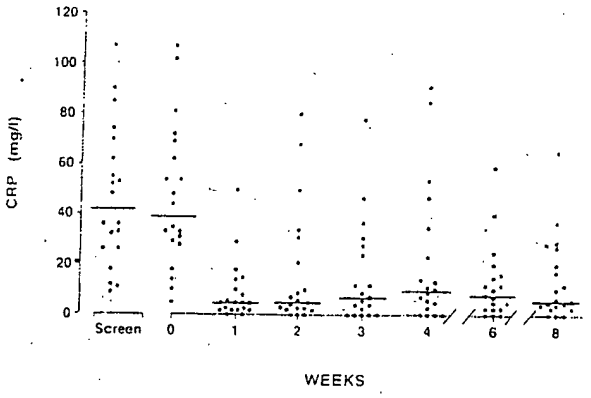

Figure 2. Serum C-reactive protein (CRP) levels (normal 0–10 mg/liter), as measured by nephelometry, in 20 patients with rheumatoid arthritis treated with cA2. The screening time point was within 4 weeks of entry to the study (week 0); data from patient 15 were not included after week 2 (dropout). Significance of the changes, relative to week 0, were determined by Mann-Whitney test (adjusted): $P < 0.001$ at week 1, $P < 0.003$ at week 2, $P < 0.002$ at week 3, $P < 0.02$ at week 4, and $P < 0.001$ at weeks 6 and 8. Bars show median values.

according to the index described by Paulus et al (35). At week 6, all patients showed a mean improvement of 30% or greater in the 6 selected measures of disease activity (see Patients and Methods), with 18 of the 19 patients showing a mean improvement of 50% or greater (data not shown).

Although the study was primarily designed to assess the short-term effects of cA2 treatment, followup clinical and laboratory data are available for those patients followed for sufficient time (n = 12). The duration of response in these patients, defined as the duration of a 30% (or greater) mean improvement in the 6 selected disease activity measures, was variable, ranging from 8 weeks to 25 weeks (median 14) (data not shown).

Comparison of the clinical and laboratory data for patients treated with 2 infusions of cA2 (each at 10 mg/kg) versus those treated with 4 infusions (each at 5 mg/kg) showed no significant differences in the rapidity or extent of response (data not shown).

Immunologic investigations and cytokines. Measurement of rheumatoid factor by RAPA showed 14 patients with significant titers (≥1:160) at trial entry. Of these, 6 patients showed a decrease of at least 2 titers on treatment with cA2, while the remaining patients showed a change of 1 titer or less. No patient showed a significant increase in rheumatoid factor titer during the trial (data not shown). The median titer in the 11 patients decreased from 1:2,560 at entry to 1:480 by week 8 ($P > 0.05$) (Table 3). Specific rheumatoid factor isotypes were measured by ELISA, and showed decreases in the 6 patients whose RAPA had declined significantly, as well as in some other patients (data not shown). Median values for the 3 isotypes in the 14 patients seropositive at trial entry were 119, 102, and 62 IU/ml (IgM, IgG, and IgA isotypes, respectively) and at week 8 were 81, 64, and 46 IU/ml ($P > 0.05$).

We tested sera from patients 1–9 for the presence of bioactive TNF, using the WEHI 164 clone 13 cytotoxicity assay (36). In 8 patients, serum samples spanning the entire trial period were tested, while for patient 9, only 3 samples (1 pretrial, 1 intermediate, and the last available sample) were tested. The levels of bioactive TNF were below the limit of sensitivity of the assay in the presence of human serum (1 pg/ml) (data not shown).

Since production of CRP and SAA are thought to be regulated in large part by IL-6, we also measured serum levels of this cytokine, using 2 different assays which measure total IL-6. In the Medgenix assay, IL-6 was significantly elevated in 17 of the 20 patients at study entry. In this group, levels fell from 60 pg/ml (range 18–500) to 40 pg/ml (range 0–230) at week 1 ($P > 0.05$) and to 32 pg/ml (range 0–210) at week 2 ($P < 0.005$ and $P < 0.01$, adjusted). These results were supported by measurement of serum IL-6 in the first 16 patients in a separate ELISA developed in-house. IL-6 was detectable in 11 of these samples, with median (range) levels falling from 210 pg/ml (25–900) at entry to 32 pg/ml (0–1,700) at week 1 ($P < 0.02$ and $P < 0.04$, adjusted) and to 44 pg/ml (0–240) at week 2 ($P < 0.02$ and $P < 0.03$, adjusted).

We tested sera from patients 1–10 for the presence of antiglobulin responses to the infused chimeric antibody, but none were detected (data not shown). In many patients, however, cA2 was still detectable in serum samples taken at week 8 (data not shown) and this may have interfered with the ELISA.

DISCUSSION

This is the first report describing the administration of anti-TNFα antibodies for treatment of human autoimmune disease. Many cytokines are produced in rheumatoid synovium, but we chose to specifically target TNFα because of mounting evidence that it was a major molecular regulator in RA (21,22,26–28). The study results presented here support that view and allow 3 important conclusions to be drawn.

First, treatment with cA2 was safe and the infusion procedure was well tolerated. Although fever, headache, chills, and hemodynamic disturbance have all been reported following treatment with anti-CD4 or anti-CDw52 in RA (6,10), such features were absent in our patients. Also notable was the absence of any allergic event despite repeated treatment with the chimeric antibody, although the interval between initial and repeat infusions may have been too short to allow maximal expression of any antiglobulin response. The continuing presence of circulating cA2 at the conclusion of the trial may have precluded detection of antiglobulin responses, but also indicated that any such responses were likely to be of low titer and/or affinity. Although we recorded 2 episodes of infection among the study group, these were minor and the clinical courses were unremarkable. TNFα has been implicated in the control of *Listeria* and other infections in mice (37), but our limited experience does not suggest an increased risk of infection after TNFα blockade in humans.

The second conclusion concerns the clinical

efficacy of cA2. The patients we treated had longstanding, erosive, and for the most part, seropositive disease, and therapy with several standard DMARDs had failed. Despite this, the major clinical assessments of disease activity and outcome (morning stiffness, pain score, Ritchie articular index, swollen joint count, and HAQ score) showed statistically significant improvement, even after adjustment for multiple comparisons. All patients graded their response as at least "fair," with the majority grading it as "good." In addition, all achieved a response according to the criteria of Paulus et al and showed a mean improvement of at least 30% in 6 selected disease activity measures. The design of the trial does not allow these results to be attributed to the action of cA2 alone. However, the extent of the clinical improvements, their consistency throughout the study group, and the parallel changes in laboratory indices of disease activity (see below) are encouraging.

The improvements in clinical assessments following treatment with cA2 appear to be at least as good as those reported following treatment of similar patients with antileukocyte antibodies (6,10), although firm conclusions concerning each of these agents will require controlled, blinded studies. The two therapeutic approaches may already be distinguished, however, by their effects on the acute-phase response, since in several studies of antileukocyte antibodies, no consistent improvements in CRP or ESR were seen (4–6,8,10). In contrast, treatment with cA2 resulted in significant decreases in serum CRP and SAA values, with normalization of values in many patients. The changes were rapid and marked, and in the case of CRP, sustained for the duration of the study (Table 3). The decreases in ESR were less marked, achieving statistical significance only when unadjusted for the number of comparisons (Table 3).

These results are consistent with current concepts that implicate TNFα in the regulation of hepatic acute-phase protein synthesis, either directly, or by control of other, secondary, cytokines such as IL-6 (38,39). To investigate the mechanism of control of the acute-phase response in our patients, we measured serum TNFα and IL-6 before and after cA2 treatment. Bioactive TNFα was not detectable in sera obtained at baseline or subsequently. In view of previous reports of variability between different immunoassays in the measurement of cytokines in biologic fluids (40), we used 2 different assays for IL-6, and both demonstrated significant decreases in serum IL-6 levels by week 2. These findings support the other objective laboratory changes induced by cA2, and provide in vivo evidence that TNFα may be a regulatory cytokine for IL-6 in this disease. Among the other laboratory tests performed, levels of rheumatoid factors fell significantly in 6 patients.

The mechanism of action of cA2 leading to the clinical responses outlined above was not established in this study. Neutralization of TNFα may have a number of beneficial consequences, including a reduction in the local release of cytokines such as IL-6 and other inflammatory mediators, and modulation of synovial endothelial/leukocyte interactions. cA2 may also bind directly to synovial inflammatory cells expressing membrane TNFα, with subsequent in situ cell lysis. Further studies should establish which actions of cA2 may be clinically important.

The results obtained in this small series have important implications, both scientifically and clinically. At the scientific level, the ability of the neutralizing antibody, cA2, to reduce acute-phase protein synthesis, reduce the production of other cytokines such as IL-6, and significantly improve the clinical state demonstrates that it is possible to interfere with the cytokine network in a useful manner without untoward effects. Due to the many functions and overlapping effects of cytokines such as IL-1 and TNFα, and the fact that cytokines induce the production of other cytokines and of themselves, there had been some pessimism as to whether targeting a single cytokine in vivo would have any beneficial effect (41,42). This view is clearly refuted. On the clinical side, the results of short-term treatment with cA2 are encouraging, and suggest that TNFα may be a useful new therapeutic target in RA.

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Inhibition of immunoreactive tumor necrosis factor-alpha by a chimeric antibody in patients infected with human immunodeficiency virus type 1.

J Infect Dis. 1996 Jul;174(1):63-8. Unique Identifier : AIDSLINE MED/96261994

Walker RE; Spooner KM; Kelly G; McCloskey RV; Woody JN; Falloon J; Baseler M; Piscitelli SC; Davey RT Jr; Polis MA; Kovacs JA; Masur H; Lane HC; National Institute of Allergy and Infectious Diseases, Critical; Care Medicine Department, National Institutes of Health,; Bethesda, MD 20892, USA.

Abstract: Tumor necrosis factor-alpha (TNF-alpha), a proinflammatory cytokine known to stimulate human immunodeficiency virus type 1 (HIV-1) replication, has been implicated in the pathogenesis of HIV-1 infection. Inhibition of TNF-alpha by a chimeric humanized monoclonal antibody, cA2, was investigated in 6 HIV-1-infected patients with CD4 cell counts < 200/mm3. Two consecutive infusions of 10 mg/kg 14 days apart were well tolerated, and a prolonged serum half-life for cA2 (mean, 257 +/- 70 h) was demonstrated. Serum immunoreactive TNF-alpha concentrations fell from a mean prestudy value of 6.4 pg/mL (range, 4.2-7.9) to 1.1 pg/mL (range, 0.5-2.2) 24 h after the first infusion and returned to baseline within 7-14 days. A similar response was seen after the second infusion. No consistent changes in CD4 cell counts or plasma HIV RNA levels were observed over 42 days. Future studies evaluating the therapeutic utility of long-term TNF-alpha suppression using anti-TNF-alpha antibodies are feasible and warranted.

Keywords: Acquired Immunodeficiency Syndrome/BLOOD/*IMMUNOLOGY Adult Animal Antibodies/*THERAPEUTIC USE Antibodies, Monoclonal/*THERAPEUTIC USE Chimeric Proteins/PHARMACOKINETICS/*THERAPEUTIC USE Female Human *HIV-1 Male Mice Recombinant Proteins/PHARMACOKINETICS/THERAPEUTIC USE Tumor Necrosis Factor/*ANTAGONISTS & INHIB/IMMUNOLOGY JOURNAL ARTICLE

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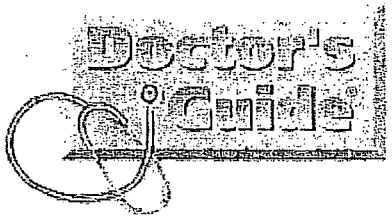

Medical & Other News

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Title: **New Monoclonal Antibody Effective Treatment For Crohn's Disease Therapy**
URL: http://www.pslgroup.com/dg/2802E.htm
Doctor's Guide
May 13, 1997

WASHINGTON, and MALVERN, Pa., May 13, 1997 -- Statistically significant results were released yesterday from two controlled clinical studies testing cA2(TM) (infliximab), a monoclonal antibody, in the treatment of Crohn's disease, a chronic disorder characterized by inflammation of the gastrointestinal tract. Data from both trials show that treatment with cA2 can have a beneficial effect on both the severity and number of symptoms associated with Crohn's disease.

"This kind of clinical response in Crohn's disease is unprecedented," said Stephan Targan, M.D., principal investigator and Director of the Inflammatory Bowel Disease Center at Cedars-Sinai Medical Center in Los Angeles, "and provides compelling evidence of the potential of cA2 in the treatment of Crohn's disease."

The results of these trials, which were conducted in 18 centers in North America and Europe, were announced today during Digestive Disease Week in Washington, DC. Digestive Disease Week is sponsored by the American Association for the Study of Liver Diseases, the American Gastroenterological Association, the American Society for Gastrointestinal Endoscopy and The Society for Surgery of the Alimentary Tract.

Last year, during Digestive Disease Week, Centocor released data showing a statistically significant improvement in disease activity following a single infusion of cA2. In the initial study, 65 percent of patients treated with cA2 achieved a clinical response and 33 percent of patients went into remission within four weeks of the start of treatment.

In the extension phase of this study, known as T16, which is being reported today, additional cA2 treatments were demonstrated to maintain Crohn's disease patients in clinical remission as measured by the CDAI, the Crohn's disease activity index.

In the initial phase of the T16 trial, the median CDAI of treated patients dropped from 312 to 125 eight weeks after a single cA2 infusion. Following four additional infusions, given eight weeks apart in the most recent phase of the T16 trial, cA2 maintained the CDAI reduction, with median CDAI eight weeks following the final treatment at 117 (CDAI<150 constitutes disease remission).

Data from the second trial, named T20, indicate that cA2 may be a valuable treatment for enterocutaneous fistulae, a painful, debilitating complication of Crohn's disease in which extensions occur between the bowel and the skin, mostly in the perianal area, causing drainage of mucous and/or fecal material. In this trial, approximately two-thirds of participants experienced closure of at least 50 percent of their fistulae.

In both clinical trials, onset of cA2 clinical benefit was rapid with the vast majority of responders achieving response within two weeks. In addition, cA2 was generally well tolerated in these two trials. "We have been following these studies with great interest," said Richard P. MacDermott, M. D., Immediate Past Chairperson, National Scientific Advisory Committee, Crohn's & Colitis Foundation of America (CCFA). "The results are very encouraging. It is possible that an important new therapy for Crohn's disease may be on the horizon."

In the T16 study, 73 patients who showed a clinical response eight weeks after their initial infusion of cA2 were re-randomized at week 12 to further treatment with cA2 or placebo, and infused every eight weeks for a total of four additional infusions. Those patients re-randomized to cA2 continued to experience an improvement in symptoms from baseline assessment and the percentage of patients achieving clinical remission was maintained at approximately 60 percent during the re-treatment period.

Those patients who responded to their initial infusion of cA2 and then received placebo in the re-treatment phase of the study, experienced a gradual decline in clinical effect over time. However, 19 percent of the placebo group were still in remission 48 weeks after their initial cA2 infusion.

The second study, T20, was conducted with 94 patients with draining enterocutaneous fistulae. Following a series of three cA2 infusions given two and four weeks apart, two-thirds of patients experienced closure of at least 50 percent of their fistulas (P=0.002). These patients had previously failed to respond adequately to treatment with combinations of corticosteroids, methotrexate, 6-MP/azathioprine, aminosalicylates, or antibiotics. These underlying therapies were given in conjunction with the cA2 infusions in this study. "cA2 is the first drug to ever demonstrate statistical significance in a controlled trial to close fistulas," according to Daniel Present, M.D., principal investigator and Clinical Professor of Medicine at Mount Sinai.

cA2, a monoclonal antibody, is the first of a revolutionary class of agents being studied for Crohn's disease. It is a well-tolerated, highly selective treatment that blocks activity of a key inflammatory mediator called tumor necrosis factor or TNF. cA2 is also being studied for treatment of rheumatoid arthritis.

Centocor is a biotechnology company whose mission is to develop and commercialize novel therapeutic and diagnostic products and services that solve critical needs in human health care. The company concentrates on research and development, manufacturing and market development, with a primary technology focus on monoclonal antibodies and DNA-based products.

More information about the company and cA2 can be found on Centocor's home page located at the following address. For more information about Crohn's disease or ulcerative colitis, a related disorder, contact the Crohn's & Colitis Foundation of America, at 1-800-343-3637 (website: http://www.ccfa.org).

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-continued

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATCGGACGTGGACGTGCAGA 20

What is claimed is:

1. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 3 and SEQ ID NO: 5.

2. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 1, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

3. A chimeric antibody comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human tumor necrosis factor TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

4. An immunoassay method for detecting human TNF in a sample, comprising:

(a) contacting said sample with an antibody according to claim 3, or a TNF binding fragment thereof, in detectably labeled form; and

(b) detecting the binding of the antibody to said TNF.

5. A chimeric antibody, comprising two light chains and two heavy chains, each of said chains comprising at least part of a human immunoglobulin constant region and at least part of a non-human immunoglobulin variable region, said variable region capable of binding an epitope of human tumor necrosis factor hTNFα, wherein said light chains comprise variable regions comprising SEQ ID NO: 3 and said heavy chains comprise variable regions comprising SEQ ID NO: 5.

6. A chimeric antibody according to claim 5, wherein the human immunoglobulin constant region is an IgG1.

7. A chimeric antibody comprising at least part of a human IgG1 constant region and at least part of a non-human immunoglobulin variable region, said antibody capable of binding an epitope specific for human TNFα, wherein the non-human immunoglobulin variable region comprises a polypeptide encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NO: 2 and SEQ ID NO: 4.

8. A polypeptide comprising the amino acid sequence of SEQ ID NO: 3, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

9. A polypeptide comprising the amino acid sequence of SEQ ID NO: 5, wherein said polypeptide binds to hTNFα and competitively inhibits the binding of monoclonal antibody cA2 to hTNFα.

* * * * *

HYBRITECH INCORPORATED, Appellant,

v.

MONOCLONAL ANTIBODIES, INC., Appellee.

Appeal No. 86-531.

United States Court of Appeals, Federal Circuit.

Sept. 19, 1986.

Suit was brought alleging infringement of patent No. 4,376,110 for immunometric assays using monoclonal antibodies. The United States District Court, Northern District of California, Samuel Conti, J., 623 F.Supp. 1344, held that patent was invalid, and patent holder appealed. The Court of Appeals, Rich, Circuit Judge, held that: (1) laboratory notebooks of patent holder were admissible to show date upon which patent holder first conceived of such assays, though notebooks were not witnessed until a few months to one year after writing; (2) patent was not invalid for obviousness; and (3) patent was not invalid for holder's alleged failure to disclose best mode of carrying out his invention.

Reversed and remanded.

1. Federal Courts ⇐848, 850

District court's findings of fact, which were almost verbatim adoption of findings of fact prepared by prevailing party in advance of trial, were nevertheless findings of court which could not be reversed unless clearly erroneous. Fed.Rules Civ.Proc. Rule 52(a), 28 U.S.C.A.

2. Patents ⇐90(5)

Date on which invention was conceived or reduced to practice is a legal determination, which is subject to review free of clearly erroneous standard. 35 U.S.C.A. § 102(g).

3. Patents ⇐90(5)

Invention is actually reduced to practice, for purposes of determining its priority under federal patent law, only when it works for its intended purpose. 35 U.S.C.A. § 102(g).

4. Evidence ⇐377

Laboratory notebooks of claimed inventor of immunometric assays using monoclonal antibodies were admissible to show date on which inventor first conceived of such assays, for purpose of inventor's patent infringement claim, even though notebooks were not witnessed until a few months to one year after their writing. 35 U.S.C.A. § 102(g).

5. Patents ⇐312(2)

Largely uncorroborated testimony of inventors was not admissible to show date on which invention was first reduced to practice, for purpose of determining whether invention qualified as prior art. 35 U.S.C.A. § 102(g).

6. Patents ⇐324.5

Whether patent is invalid for obviousness constitutes legal determination, which is reviewed free of clearly erroneous standard. 35 U.S.C.A. § 103.

7. Patents ⇐26(2), 36.1(3, 4), 36.2(1)

In determining whether patent is invalid for obviousness, court must consider objective evidence such as patented invention's commercial success, long-felt need for invention, failure of other inventions, and unexpected results of invention. 35 U.S.C.A. § 103.

8. Patents ⇐16.33

Patent No. 4,376,110 for immunometric assays using monoclonal antibodies was not invalid for obviousness, where patented invention enjoyed immediate commercial success not attributable solely to advertising, led to unexpected advances in area of medical diagnosis, and was in no way suggested by scientists' use of monoclonal antibodies of different affinity. 35 U.S.C.A. § 103.

9. Patents ⇐99

Determination that patent enables one skilled in art to make and use claimed invention is not precluded, so that patent is

not necessarily invalid, even though some experimentation is necessary. 35 U.S.C.A. § 112.

10. Patents ⇐99

Whether patent enables one skilled in art to make and use claimed invention should be determined, for purpose of evaluating validity of patent, based on information available to those skilled in art when patent application was filed. 35 U.S.C.A. § 112.

11. Patents ⇐99

Patent No. 4,376,110 for immunometric assays using monoclonal antibodies contained sufficient disclosures to permit one skilled in art to make and use invention, so that patent was not on that basis invalid. 35 U.S.C.A. § 112.

12. Patents ⇐99

To show that patent is invalid for failing to comply with best mode requirement, challenger must show that applicant knew of and concealed better mode of carrying out his invention. 35 U.S.C.A. § 112.

13. Patents ⇐312(6)

Evidence that holder of patent for immunometric assays using monoclonal antibodies employed sophisticated competent people to perform screening, and that screening process was labor-intensive and time-consuming, was not sufficient to show that holder knowingly concealed best mode of screening or producing monoclonal antibodies for use in patent process. 35 U.S.C.A. § 112.

14. Patents ⇐99

Patent is not invalid for indefiniteness, where patent reasonably apprises those skilled in art both of utilization and scope of invention, and language is as precise as subject matter permits. 35 U.S.C.A. § 112.

Douglas E. Olson, Lyon & Lyon, Los Angeles, Cal., for appellant. With him on brief were James W. Geriak and Bradford J. Duft.

David J. Brezner, Flehr, Hohback, Test, Albritton & Herbert, San Francisco, Cal., for appellee. Barry E. Bretschneider and Herbert I. Cantor, Washington, D.C., of counsel.

Before RICH, DAVIS and SMITH, Circuit Judges.

RICH, Circuit Judge.

This appeal is from the August 28, 1985, decision of the United States District Court for the Northern District of California, 623 F.Supp. 1344, 227 USPQ 215, in favor of defendant Monoclonal Antibodies, Inc. (Monoclonal) holding that all 29 claims of plaintiff's patent No. 4,376,110 entitled "Immunometric Assays Using Monoclonal Antibodies" ('110 patent), issued to Dr. Gary S. David and Howard E. Greene and assigned to Hybritech Incorporated (Hybritech), are invalid as anticipated under 35 U.S.C. § 102(g), for obviousness under § 103, and under § 112 first and second paragraphs. We reverse and remand.

Background

Vertebrates defend themselves against invasion by microorganisms by producing antibodies, proteins which can complex with the invading microorganisms and target them for destruction or removal. In fact, any foreign molecule of sufficient size can act as a stimulus for antibody production. Such foreign molecules, or antigens, bear particular sites or epitopes that represent antibody recognition sites. B cell lymphocytes, the cells that actually produce antibodies, recognize and respond to an epitope on an antigen by reproducing or cloning themselves and then producing antibodies specific to that epitope. Even if the antigen is highly purified, the lymphocytes will produce antibodies specific to different epitopes on the antigen and so produce antibodies with different specificities. Furthermore, because the body is exposed to many different antigens, the blood of a vertebrate will contain antibodies to many different antigenic substances.

Scientists and clinicians have long employed the ability of antibodies to recognize and complex with antigens as a tool to

identify or label particular cells or molecules and to separate them from a mixture. Their source of antibodies has been primarily the serum separated from the blood of a vertebrate immunized or exposed to the antigen. Serum, however, contains a mixture of antibodies directed to numerous antigens and to any number of epitopes on a particular antigen. Because such a mixture of antibodies arises from many different clones of lymphocytes, it is called "polyclonal."

Recent technological advances have made it possible to isolate and cultivate a single clone of lymphocytes to obtain a virtually unlimited supply of antibodies specific to one particular epitope. These antibodies, known as "monoclonal antibodies" because they arise from a single clone of lymphocytes, are produced by a relatively new technology known as the hybridoma. Hybridomas are produced by fusing a particular cancer cell, the myeloma cell, with spleen cells from a mouse that has been injected or immunized with the antigen. These fusions are isolated by transferring them to a growth fluid that kills off the unfused cancer cells, the unfused spleen cells dying off by themselves. The fused hybrid spleen and myeloma cells, called hybridomas, produce antibodies to the antigen initially injected into the mouse. The growth fluid containing the hybridomas is then diluted and put into individual test tubes or wells so that there is only one hybridoma per tube or well. Each hybridoma then reproduces itself and these identical hybridomas each produce identical monoclonal antibodies having the same affinity and specificity. In this way, a virtually unlimited supply of identical antibodies is created, directed to only one epitope on an antigen rather than, as with polyclonal antibodies, to many different epitopes on many different antigens.

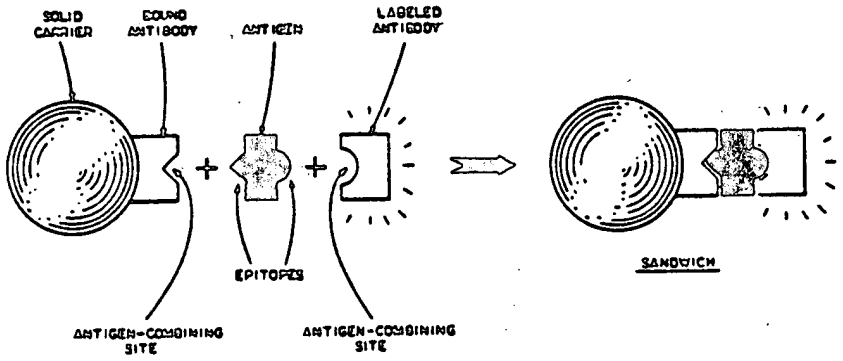
In addition to the specificity of antibodies to particular epitopes discussed above, antibodies also have a characteristic "sensitivity," the ability to detect and react to antigens. Sensitivity is expressed in terms of "affinity:" the greater an antibody's ability to bind with a particular antigen, the greater the antibody's affinity. The strength of that antibody-antigen bond is in part dependent upon the antibody's "affinity constant," expressed in liters per mole, for the antigen.

Immunoassays, the subject matter of the '110 patent, are diagnostic methods for determining the presence or amount of antigen in body fluids such as blood or urine by employing the ability of an antibody to recognize and bind to an antigen. Generally, the extent to which the antibody binds to the antigen to be quantitated is an indication of the amount of antigen present in the fluid. Labelling the antibody or, in some cases, the antigen, with either a radioactive substance, I^{125}, or an enzyme makes possible the detection of the antibody-antigen complex. In an extreme case, where the fluid sample contains a very low level of the antigen, binding might not occur unless the antibodies selected or "screened" for the procedure are highly sensitive.

In the case of a "competitive" immunoassay, a labelled antigen reagent is bound to a limited and known quantity of antibody reagent. After that reaction reaches equilibrium, the antigen to be detected is added to the mixture and competes with the labelled antigen for the limited number of antibody binding sites. The amount of labelled antigen reagent displaced, if any, in this second reaction indicates the quantity of the antigen to be detected present in the fluid sample. All of the antigen attached to the antibody will be labelled antigen if there is no antigen in the test fluid sample. The advantage of this method is that only a small amount of antibody is needed, its drawback, generally, that the system must reach equilibrium, and thus produces results slowly.

In the case of a "sandwich" assay, otherwise known as an immunometric assay, the latter being a term coined by Dr. Lawton Miles in 1971, a quantity of unlabelled antibody reagent is bound to a solid support surface such as the inside wall of a test tube containing a complex of the fluid sam-

ple containing the antigen to be detected and a labelled *antibody* reagent. The result is an insoluble three part complex referred to as a sandwich having antibody bread and antigen filling. This figure is illustrative of the sandwich concept:



The advantage of the sandwich assay is that it is fast and simple, its drawback that enormous quantities of antibodies are needed.

Hybritech

Hybritech, started in 1978 and joined thereafter by coinventors Green and Dr. David, has, since 1979, been in the business of developing diagnostic kits employing monoclonal antibodies that detect numerous antigens and thus a broad range of conditions such as pregnancy, cancer, growth hormone deficiency, or hepatitis. Examples of antigens include influenza viruses, immunoglobulin E (IgE) which indicates allergic reaction, human chorionic gonadotropin (HCG) which indicates pregnancy, and prostatic acid phosphatase (PAP) which indicates prostate cancer, to name a few. Dr. Adams, a business-experienced scientist, joined the company in May 1980 as head of research and development. The '110 patent, application for which was filed August 4, 1980, issued March 8, 1983, with claims defining a variety of sandwich assays using monoclonal antibodies. Claim 19, apparently the broadest of the twenty-nine in the patent, is directed generally to a sandwich assay and reads (emphasis ours):

> 19. *In an immunometric assay* to determine the presence or concentration of an antigenic substance in a sample of a fluid comprising forming a ternary complex of a first labelled antibody, said antigenic substance, and a second antibody said second antibody being bound to a solid carrier insoluble in said fluid wherein the presence of the antigenic substance in the samples is determined by measuring either the amount of labelled antibody bound to the solid carrier or the amount of unreacted labelled antibody, *the improvement comprising* employing monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole for each of said labelled antibody and said antibody bound to a solid carrier.

Claim 1, directed particularly to a reverse sandwich assay, explained infra, reads:

> 1. A process for the determination of the presence of [sic, or] concentration of an antigenic substance in a fluid comprising the steps:
>
> (a) contacting a sample of the fluid with a measured amount of a soluble first monoclonal antibody to the antigenic substance in order to form a soluble complex of the antibody and antigenic substance present in said sample, said first monoclonal antibody being labelled;
>
> (b) contacting the soluble complex with a second monoclonal antibody to the antigenic substance, said second monoclonal antibody being bound to a solid carrier, said solid carrier being

insoluble in said fluid, in order to form an insoluble complex of said first monoclonal antibody, said antigenic substance and said second monoclonal antibody bound to said solid carrier;

(c) separating said solid carrier from the fluid sample and unreacted labelled antibody;

(d) measuring either the amount of labelled antibody associated with the solid carrier or the amount of unreacted labelled antibody; and

(e) relating the amount of labelled antibody measured with the amount of labelled antibody measured for a control sample prepared in accordance with steps (a)-(d), said control sample being known to be free of said antigenic substance, to determine the presence of antigenic substance in said fluid sample, or relating the amount of labelled antibody measured with the amount of labelled antibody measured for samples containing known amounts of antigenic substance prepared in accordance with steps (a)-(d) to determine the concentration of antigenic substance in said fluid sample, the first and second monoclonal antibodies having an affinity for the antigenic substance of at least about 10^8 liters/mole.

The District Court Decision

Hybritech sued Monoclonal March 2, 1984, for damages and an injunction alleging that the manufacture and sale of Monoclonal's diagnostic kits infringed the '110 patent. Trial without a jury began on August 5, 1985, and concluded August 23, 1985, thirty witnesses having been heard and over 2,000 pages of transcript generated. The district court produced the reported opinion, findings, and conclusions, which use nearly verbatim Monoclonal's *pre-trial* brief and *pre-trial proposed* findings of fact and conclusions of law, in three days, in support of the judgment now on appeal.

The district court held that the claimed subject matter of the '110 patent was neither conceived nor actually reduced to practice before May 1980, and was anticipated under § 102(g) by the actual reduction to practice of the invention by Drs. Uotila and Ruoslahti at the La Jolla Cancer Research Foundation (LJCRF) as early as November of 1979 and by the actual reduction to practice of the invention by Drs. Oi and Herzenberg (Oi/Herzenberg work) at the Stanford University Laboratory as early as July 1978, later published in December of 1979.

The district court also held the claims of the '110 patent invalid for obviousness from the Oi/Herzenberg work in view of (1) a February 1979 article by M.E. Frankel and W. Gerhard (Frankel article) which discloses high-affinity monoclonal antibodies, and apparently in view of numerous other references including; (2) the work of Nobel Prize winners G. Kohler and C. Milstein disclosing a Nobel Prize-worthy method for producing monoclonal antibodies in vitro (outside the body) published in an August 7, 1975, article; (3) U.S. Patent No. 4,244,940 issued to Jeong et al. disclosing a simultaneous polyclonal assay (Jeong), U.S. Patent No. 4,098,876 to Piasio et al. disclosing a reverse polyclonal sandwich assay (Piasio), U.S. Patent No. 4,016,143 to Schurrs et al. disclosing a forward polyclonal sandwich assay (Schurrs); (4) a July 1979 publication by A.C. Cuello et al. disclosing the use of monoclonal antibodies in competitive assays; and (5) eight articles dated between January 1979 and March 6, 1980, "predicting" that monoclonal antibodies would be used in future immunoassays.[1]

The district court also invalidated the patent on various grounds based on 35 U.S.C. § 112, first and second paragraphs, as hereinafter discussed.

1. With respect to obviousness, one portion of the district court's opinion apparently relies on all of the above listed references, (1)—(5), for the obviousness holding while a later portion entitled "CONCLUSIONS OF LAW" relies on only the Oi/Herzenberg and Frankel articles. Furthermore, the district court did not state that the LJCRF work was considered for purposes of § 103, although we recognize that § 102(g) prior art can be used for § 103.

A. *The References*

1. *Kohler and Milstein's Nobel Prize-Winning Work: Producing Monoclonal Antibodies In Vitro For the First Time*

In early immunoassay work, polyclonal antibodies produced in vivo (in the body) in mice were used to bind with the antigen to be detected in the body fluid sample. Mice were immunized by injection with antigen so that the lymphocytes in their bodies produced antibodies that attacked the injected antigen. Those polyclonal antibodies were withdrawn from the animal's blood and used in immunoassays. The major problem was that when the mice's immune systems changed or the mice died, the antibodies changed or died too; supply was limited and uncertain.

As the examiner was aware, Kohler and Milstein developed a technique not only for producing antibodies in vitro, independent of a living body, thus eliminating dependence on a particular animal, but for in vitro production of monoclonal antibodies by hybridomas, discussed in the Background section, supra.

Given that sandwich assays require enormous amounts of antibodies, companies like appellant and appellee, which utilize monoclonal antibodies for sandwich assays, would not be in business were it not for the work of Kohler and Milstein.

2. *The Work of Drs. Ruoslahti, Uotila, and Engvall at the La Jolla Cancer Research Foundation (LJCRF) in 1979 and 1980*

Dr. Ruoslahti performed mostly competitive immunoassays using polyclonal antibodies to alphafetoprotein (AFP) antigens at the City of Hope since 1970. Dr. Uotila joined him in late 1978 to perform immunoassays using monoclonal antibodies to AFP. After producing monoclonal antibodies to AFP and performing competitive radio immunoassays (RIA—a competitive assay that uses a radioactive label) with monoclonal antibodies at the City of Hope in mid–1979, Drs. Ruoslahti, Uotila and Engvall left LJCRF.

In the fall of 1979, September or October according to Dr. Uotila, discussion and work began on using monoclonal antibodies to AFP in a sandwich assay. Dr. Uotila, the principal researcher in this particular endeavor, generated six notebooks while at the City of Hope and LJCRF. The next-to-last page of notebook four contained a note to Dr. Uotila from Dr. Ruoslahti reading:

> Sometime you should enzyme label a good monoclonal antibody so that you can set up a sandwich assay. If you use two monoclonal antibodies, you may be able to do the assay with a single incubation, since the monoclonal antibodies are likely to be directed against different determinants and not compete with one another.

Although Dr. Uotila's notebook pages were, for the most part, unsigned, undated, and uncorroborated, Dr. Ruoslahti's testimony, placed the date of this note at about October 1979 by referring to the first pages of notebook five which were dated in early November 1979. Dr. Ruoslahti testified that one curve on one graph on page 43D of notebook five showed a successful simultaneous sandwich assay using monoclonal antibodies about November 5, 1979, although no data supporting that graph could be found elsewhere in the notebook. He further testified that the affinity of the monoclonal antibodies used for that test was not calculated until 1980 but that the raw data necessary for that calculation was generated in 1979.

Dr. Uotila stated in her deposition (she did not testify at trial) that she started work on a sandwich assay using monoclonal antibodies between October 4 and the end of that month, 1979, and that she could not remember the procedure used nor was there enough information in her notebook, including page 43D, to refresh her memory. She did remember, although she continued work on this assay because the tests did not yield repeatedly good curves without which she would not publish her work, that the assay on page 43D was successful. Dr. Engvall testified about a discussion of Dr. Uotila's monoclonal antibody work with

her while at the City of Hope and about first performing a sandwich assay after arriving at LJCRF in 1979.

3. *The Work of Drs. Oi and Herzenberg at the Stanford University Laboratory in 1978 Published in December 1979*

Drs. Oi and Herzenberg used monoclonal antibodies to "map" epitopes or determine the number and location of different antibody binding sites on a known quantity of IgE antigen by attaching to it an antibody bound to a carrier and exposing that antigen to other monoclonal antibodies. The antibodies either attached to epitopes on the antigen or were blocked from doing so by the other monoclonal antibodies, depending on the location and number of epitopes; if the epitopes on the antigen were too close together and the number of antibodies too great, few antibodies would bind to the antigen. Hybritech points out that both Dr. Herzenberg and Dr. Oi testified that *their work did not involve determining the presence or quantity of antigen,* that they had no idea what the affinities of the monoclonal antibodies used were, and that those values were never calculated.

One unsigned, unwitnessed page from three large laboratory notebooks, which Hybritech argues is insufficient because it does not identify the chemical reagents or protocol used, was relied on by Monoclonal to establish actual reduction to practice of the Oi/Herzenberg work in 1978 to establish a case of § 102(g) prior invention by another. The district court agreed with Monoclonal that the Oi/Herzenberg work anticipated the claimed invention and, in addition, combined this work with the Frankel publication to hold that the claimed subject matter was obvious under § 103.

4. *The Frankel Article: Monoclonal Antibodies Having Affinities of 10^9 liters/mole*

Frankel describes an RIA (radioimmunoassay) method for the rapid determination of affinity constants for monoclonal antibodies produced from hybridomas. The article states that the assay used is applicable only to antibodies with binding constants of about 10^{10} liters/mole and discloses the binding constants for antibodies to several closely related strains of influenza virus.

The district court found that Frankel disclosed monoclonal antibodies having the affinity constants claimed in the '110 patent, 10^8 to over 10^9 liters/mole.

5. *The Cuello Article and the Jeong, Piasio, and Schurr Patents Considered by the Examiner*

Cuello, dated July 1979, states that it describes the usefulness of monoclonal antibodies in the characterization and localization of neurotransmitters such as Substance P, a peptide clearly associated with the transmission of primary sensory information in the spinal cord. The article discloses producing monoclonal antibodies from hybrid myelomas (hybridomas), their use in conventional radioimmunoassay techniques, and the benefits from doing so which flow from the ability to derive permanent cell lines capable of continuous production of highly specific antibodies.

The district court found that the examiner twice rejected all of the claims of the '110 patent based on Cuello alone or in combination with the Jeong, Piasio, and Schurr references which disclose various sandwich assays using polyclonal antibodies. The court also found that the examiner allowed the claims after they were amended to include the 10^8 affinity limitation and after Richard Bartholomew, a Hybritech employee, submitted an affidavit alleging the advantages of using monoclonal rather than polyclonal antibodies in sandwich assays.

Apparently based on the testimony of Monoclonal's expert witness Judith Blakemore, a named inventor of the Jeong patent, manager of antibody programs at Bio-Rad Laboratories from 1975 to 1982, and currently manager of monoclonal antibody therapeutics at Cetus Corporation, a Hybritech competitor in immunoassay diagnostics, the district court stated that the "reasons for allowance were not well-founded because (1) the alleged advantages were

expected as naturally flowing from the well-known natural characteristics of monoclonal antibodies ...; (2) ... were not significant ...; or (3) were at best minor," although they were "argued to the examiner as if they were" important. These were Monoclonal's words from its pretrial submission adopted by the court.

6. *The References That "Predicted" the Use of Monoclonal Antibodies in Immunoassays*

The district court stated, again in Monoclonal's words, that "it is of the utmost importance" that the advantages of monoclonal antibodies were "predicted by a number of authorities," eight to be exact, not important enough to list here, after the Kohler and Milstein discovery and after monoclonal antibodies became available.

B. *The Claimed Subject Matter of the '110 Patent*

Hybritech argues that the district court's determination that there is no credible evidence of conception or reduction to practice of the '110 invention before May 1980 is error because Dr. David's laboratory notebooks, Nos. 21 and 24, clearly show successful sandwich assays using monoclonal antibodies in August, September, and October of 1979. At the least, argues Hybritech, the invention was conceived in January of 1979, long before Drs. Ruoslahti, Engvall, and Uotila began work on a sandwich assay using monoclonal antibodies, and diligence was thereafter exercised until constructive reduction to practice occurred by the filing of the '110 patent application on August 4, 1980.

Dr. David and Greene testified that pages 2118 to 2122 of Dr. David's notebook, dated January 4, 1979, and witnessed January 30, 1979, disclose the generic conception of the invention in the context of the physical support structure used to carry out a sandwich assay, and Dr. David testified on redirect that (1) Page 1128 of notebook 21, dated May 27, 1979, recorded an early attempt at a sandwich assay that failed, (2) on August 3, 1979, as recorded at page 1166, a sandwich assay using monoclonal antibody 068 attached to a solid carrier, a radio-labelled 068 antibody, and a hepatitis antigen from an Abbott Labs polyclonal competitive assay kit was successfully performed, and (3) a sandwich assay using a bound 259 antibody, a radio-labelled 068 antibody, and a hepatitis antigen was successfully performed on September 21, 1979. Hybritech also urges that work in October 1979 directed to determining whether certain monoclonal antibodies were recognizing the same or different determinants, was a reduction to practice.

Monoclonal points out that these notebook pages do not expressly state that monoclonal antibodies of 10^8 liters/mole affinity were used in a sandwich assay and that the May, August, and September notebook entries were not witnessed until about the time Dr. Adams, experienced in patent matters, joined Hybritech and advised its researchers on properly recording laboratory work. They therefore claim that actual reduction to practice was not shown before May 1980.

OPINION

I. *Review Under Rule 52(a) Fed.R.Civ.P.*

[1] Rule 52(a) "ensures care in the preparation of an opinion ... and provides appellate courts with the benefit of the District Court's insights into a case," *Pentec, Inc. v. Graphic Controls Corp.*, 776 F.2d 309, 318, 227 USPQ 766, 772 (Fed.Cir. 1985) (Harvey, Senior District Judge, concurring) by requiring a district court to "find the facts specially and state separately its conclusions of law thereon." With the exception of the first eight paragraphs, the first half of the district court's opinion here is Monoclonal's *pretrial* brief and the last three pages of the opinion are Monoclonal's *pretrial* findings of fact and conclusions of law. The district court adopted the above documents virtually verbatim, with the exception of portions of each concerning inequitable conduct and noninfringement, apparently without inviting a response from Hybritech, resulting in a repetitious (as the district court admitted in

the opinion), sometimes internally inconsistent, and hard to follow opinion that presents us with a difficult task in gleaning the basis for many of the conclusions. For some of the findings, submitted before trial, no supporting evidence was introduced at trial.

The Supreme Court, in *Anderson v. City of Bessemer City, N.C.*, 470 U.S. 564, 105 S.Ct. 1504, 84 L.Ed.2d 518 (1985), strongly criticized the practice of "verbatim adoption of findings of fact prepared by prevailing parties, particularly when those findings have taken the form of conclusory statements unsupported by citation to the record." *Anderson*, supra, 105 S.Ct. at 1511. This court also has cautioned against the adoption of findings, especially when proposed by a party before trial, as here, and stated that the likelihood of clear error in those findings increases in such a situation. *Lindemann Maschinenfabrik v. American Hoist and Derrick*, 730 F.2d 1452, 1457, 221 USPQ 481, 485 (Fed.Cir. 1984). Notwithstanding our misgivings about whether the findings in this case, prepared before any evidence was introduced, satisfy the objectives of Rule 52(a) —a carefully prepared opinion providing the reviewing court with the benefit of the district court's *reasoned insights* into the case—those findings are the district court's and may be reversed only if clearly erroneous. *See Anderson*, supra, 105 S.Ct. at 1511; *Lindemann*, 730 F.2d at 1457, 221 USPQ at 485.

"A finding is clearly erroneous when, although there is evidence to support it, the reviewing court on the entire evidence is left with the definite and firm conviction that a mistake has been committed." *United States v. United States Gypsum Co.*, 333 U.S. 364, 395, 68 S.Ct. 525, 542, 92 L.Ed. 746 (1948). "This standard plainly does not entitle a reviewing court to reverse the finding of the trier of fact simply because it is convinced that it would have decided the case differently." *Anderson*, supra, 105 S.Ct. at 1511. In other words, "if the district court's account of the evidence is plausible in light of the record viewed in its entirety" or "where there are two permissible views of the evidence," the factfinder cannot be clearly erroneous. *Anderson*, supra, at 1511 (quoting *United States v. Yellow Cab Co.*, 338 U.S. 338, 342, 70 S.Ct. 177, 179, 94 L.Ed. 150 (1949)). This is so, stated the Court in dictum, *see Anderson*, supra, 105 S.Ct. at 1516 (Blackmun, J., concurring), even when the district court's findings rest on physical or documentary evidence or inferences from other facts and not on credibility determinations. *See also* Rule 52(a) Fed.R.Civ.P. (as amended Aug. 1, 1985). If the latter are involved, "Rule 52 demands even greater deference to the trial court's findings" but a trial judge may not "insulate his findings from review by denominating them credibility determinations"; if documents or objective evidence contradict the witness's story, clear error may be found even in a finding purportedly based on a credibility determination. *Anderson*, supra, at 1512–13. We proceed in light of all these principles.

II. *Presumption of Validity*

Under 35 U.S.C. § 282, a patent is presumed valid, and the one attacking validity has the burden of proving invalidity by clear and convincing evidence. *See, e.g., American Hoist & Derrick Co. v. Sowa & Sons, Inc.*, 725 F.2d 1350, 1360, 220 USPQ 763, 770 (Fed.Cir.1984). Notwithstanding that the introduction of prior art not before the examiner may facilitate the challenger's meeting the burden of proof on invalidity, the presumption remains intact and on the challenger throughout the litigation, and the clear and convincing standard does not change. *See, e.g., Jervis B. Webb Co. v. Southern Systems, Inc.*, 742 F.2d 1388, 1392 & n. 4, 222 USPQ 943, 945 & n. 4 (Fed.Cir.1984). The only indication that the district court recognized the presumption of validity and its proper application was its statement that "[t]he key issue in this case is whether the defendant has overcome the presumption of nonobviousness." That statement, however, speaks only part of the truth; the presumption of validity goes to validity of the patent in relation to the patent statute *as a whole*, not just to nonobviousness under section 103.

III. *Prior Invention of Another, 35 U.S.C. § 102(g)*

Section 102(g) states that a person shall be entitled to a patent unless "before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it." Section 102(g) "relates to prior inventorship by another in this country" and "retains the rules governing the determination of priority of invention...." *Kimberly-Clark Corp. v. Johnson & Johnson*, 745 F.2d 1437, 1444, 223 USPQ 603, 606 (Fed.Cir.1984) (quoting P.J. Federico, *Commentary on the New Patent Act*, 35 USCA page 1, at 19 (1954)). Section 102(g) says: "In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other."

[2] Reduction to practice, and conception as well, is a legal determination subject to review free of the clearly erroneous standard. *Barmag Barmer Maschinenfabrik AG v. Murata Machinery, Ltd.*, 731 F.2d 831, 837, 221 USPQ 561, 565–66 (Fed. Cir.1984); *D.L. Auld Co. v. Chroma Graphics Corp.*, 714 F.2d 1144, 1151, 219 USPQ 13, 18 (Fed.Cir.1983). Findings of fact supporting that legal conclusion are, of course, reviewed under the clearly erroneous standard.

[3] Conception is the "formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in practice." 1 *Robinson On Patents* 532 (1890); *Coleman v. Dines*, 754 F.2d 353, 359, 224 USPQ 857, 862 (Fed.Cir.1985). Actual reduction to practice requires that the claimed invention work for its intended purpose, *see, e.g., Great Northern Corp. v. Davis Core & Pad Co.*, 782 F.2d 159, 165, 228 USPQ 356, 358, (Fed.Cir.1986), and, as has long been the law, constructive reduction to practice occurs when a patent application on the claimed invention is filed. *Weil v. Fritz*, 572 F.2d 856, 865 n. 16, 196 USPQ 600, 608 n. 16 (CCPA 1978) (citing with approval *Automatic Weighing Machine Co. v. Pneumatic Scale Corp.*, 166 F. 288 (1st Cir.1909)).

After a review of the record in its entirety, including the numerous corroborating Hybritech laboratory notebooks, internal documents, and pertinent testimony, we hold clearly erroneous the district court's finding that there is no clear or corroborated evidence "with regard to when before May 1980, the idea of actually using monoclonals in sandwich assays" was conceived or, more properly, or when the *claimed invention* was conceived, and therefore reverse the court's holding, as a matter of law, that Hybritech's inventors did not conceive the claimed invention before May 1980.

Hybritech's claim of conception, generally, is evidenced by the sometimes sparsely documented work of a start-up company whose first small advances evolved into the myriad activities of a mature company with efforts directed toward developing the claimed invention by first employing the Kohler and Milstein technology to produce the necessary monoclonal antibodies and using those antibodies in diagnostic sandwich assay kits. There is no doubt that exploiting monoclonal antibodies for use in sandwich assays was one of the major objectives of Hybritech. In a letter to Pharmacia Fine Chemicals dated April 26, 1979, Greene, in responding to Pharmacia's interest in Hybritech's products, outlined the latter's "efforts to bring the exciting new hybridoma technology into routine medical use" and its exploration of "several intriguing concepts for which monoclonals may open up new immunodiagnostic techniques heretofore infeasible with animal serums." Although company minutes in early 1979 contain little about the claimed subject matter and some of the discussions thereon, such as Greene's and Dr. Adams' conversation about monoclonal sandwich assays when the former was trying to woo Dr. Adams to join Hybritech were unrecorded, the Hybritech laboratory notebooks and the

nature of Hybritech's research program fully corroborate the testimonial evidence of conception and thus clearly support our holding that Hybritech conceived the claimed invention before LJCRF.

Dr. David's January 1979 notebook describes, in detail, as explained by Greene and Dr. David at trial, a nylon apparatus that undoubtedly could be used for performing a sandwich assay using monoclonal antibodies, although Dr. David testified on cross-examination that at that time Hybritech had not yet developed any monoclonal antibodies, including attaching one of the reagents to a solid carrier ring, contacting that ring with a fluid sample in a microtiter plate well, adding a labelled reagent to the well after rinsing, and then "counting" or measuring the amount of either the labelled or unlabelled reagent after a prescribed time and second rinsing. The notebook then describes the procedure for detecting an antibody "(a–x)" to an antigen "(x)" complete with diagrams and text, both illuminated by Dr. David at trial. The notebook further states, "Alternatively, if one wished to quantitate an antigen, y, the identical procedure would be followed, except that reagents would be reversed, i.e. the reaction would be:" and there follows a clear illustration of an antibody attached to a solid carrier reacting with an antigen to form a complex, and that complex reacting with a second labelled antibody. The notebook was signed by Dr. David on January 4, 1979, and witnessed and signed on January 30 of the same year by Dr. Curry, the first cell biologist hired at Hybritech to set up the hybridoma production program.

Dr. David testified on direct that monoclonal antibodies were developed in the following months: antigens were purchased from outside sources and purified before being injected into mice; the spleen cells from those mice were fused with myelomas; and the resultant hybridomas were separated into well plates for development, and a radioimmunoassay procedure was carried out to determine the affinity of the antibodies.

The May 1979 failed sandwich assay, witnessed in May 1980, corroborates Dr. David's testimony that a polyclonal antibody bound to a solid carrier and a labelled monoclonal antibody were used in a sandwich assay with an antigen from Abbott Labs' Ausria polyclonal diagnostic kit for hepatitis. No binding was detected.

Dr. David testified about the experiment documented in the August 1979 notebook, a sandwich assay with a hepatitis antigen from an Abbott Labs Ausria kit with two Hybritech 068 monoclonal antibodies, one attached to a solid carrier bead and the other labelled; the purpose of the experiment was to quantitate the antigen. The notebook corroborates Dr. David's testimony that the test was positive and lists the counts per minute of the labelled antibody. Defendant Monoclonal's expert Ciotti testified about this experiment:

> Also, of course, it is limited to—it is limited to hepatitis antigen. And without a generic conception, it would just be merely a—if it did work for its intended purpose—which I would assume for purposes of discussion—*it would be a reduction to practice of one embodiment.* And without a corresponding generic conception, I don't think it would be held to be the making of the invention in terms of, for instance, in claim 19. [Emphasis ours.]

Dr. David further testified that the September 21, 1979, record in David's notebook, witnessed months later, shows a reverse sandwich assay using a bound 259 monoclonal antibody and a labelled 068 monoclonal antibody with a hepatitis antigen with results confirmed by a dose response curve.[2] Hybritech further alleges that a laboratory notebook page dated October 1979 is a reduction to practice of the

2. A dose response curve is antigen concentration plotted against the signal produced by labelled antibody in an immunoassay. The signal increases with increasing antigen concentration in a successful assay but at some point decreases when the antigen concentration becomes too high.

claimed invention but fails to cite any related testimony or other evidence in support thereof.

Finally, the record shows that the claimed affinity limitation "of at least about 10^8 liters/mole" was determined and appreciated during the course of the development of the claimed subject matter. Dr. David and Dr. Adams separately testified that the screening procedures used by Hybritech ensured that only monoclonal antibodies having at least 10^8 liters/mole affinity would be used in assays. An October 1979 internal memorandum from Greene to the staff states, "To improve comparisons we will express all affinities to the base ten to the eighth which represents the lower end of the useable range."

[4] We are left with the definite and firm conviction that a mistake has been committed because the district court's account of the evidence that "there was no credible evidence of conception before May 1980" is insupportable. There is such evidence. The laboratory notebooks, alone, are enough to show clear error in the findings that underlie the holding that the invention was not conceived before May 1980. That some of the notebooks were not witnessed until a few months to one year after their writing does not make them incredible or necessarily of little corroborative value. Admittedly, Hybritech was a young, growing company in 1979 that failed to have witnesses sign the inventors' notebooks contemporaneously with their writing. Under a reasoned analysis and evaluation of all pertinent evidence, however, we cannot ignore that Hybritech, within a reasonable time thereafter, prudently had researchers other than those who performed the particular experiments witness the notebooks in response to Tom Adams' advice. The notebooks clearly show facts underlying and contemporaneous with conception of the claimed invention and in conjunction with the testimony of Dr. David and Greene, and others, are altogether legally adequate documentary evidence, under the law pertaining to conception, of the formation in the minds of the inventors of a definite and permanent idea of the complete and operative invention as it was thereafter applied in practice. We thus are not moved by Monoclonal's argument that the findings of fact underlying conception are based on credibility determinations and are more sacrosanct than usual. *See Anderson*, supra, 105 S.Ct. at 1512–13.

1. *LJCRF Is Not Prior Art*

Hybritech laboratory notebooks and the uncontradicted testimony of Dr. David and Mr. Greene show that development of the claimed invention proceeded diligently through the rest of 1979 and 1980, there being absolutely no evidence of record nor even argument by Monoclonal that Hybritech was not diligent in its efforts to reduce to practice the claimed invention during the period January 1979 to the '110 application filing date of August 4, 1980. We therefore hold as a matter of law that Hybritech's conception, which was before LJCRF conceived the claimed invention, coupled by diligence to its constructive reduction to practice by the filing of the '110 application, entitle Hybritech to priority over LJCRF. *See* 35 U.S.C. § 102(g). The work of LJCRF is therefore not prior art.

[5] We also note that there is inadequate factual basis for the district court's holding that LJCRF reduced the claimed invention to practice as early as November 1979 because the only evidence that corroborates the testimony of Ruoslahti, Uotila, and Engvall is the note from Ruoslahti to Uotila, see section A, 2, supra, which indisputably is not the claimed invention, and the *one* curve from *one* graph from only one page, 43D, of the six Uotila notebooks. After a reasoned examination, analysis, and evaluation of this pertinent evidence we conclude that it falls far short of showing the "formation in the mind of the inventor, of a definite and permanent idea of the complete and operative invention, as it is hereafter to be applied in practice," *see Coleman*, 754 F.2d at 359, 224 USPQ at 862, and therefore is legally inadequate to support even a holding of *conception* of

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the claimed invention by LJCRF personnel in 1979.

(1) It is undisputed that page 43D was not signed, witnessed, or dated; (2) the deposition testimony of Uotila was that she could not remember the procedure used to arrive at the dose-response curve on page 43D and there was not enough information in her notebook to refresh her memory; (3) the testimony of Ruoslahti was that he could find *no* data in the notebook supporting that graph, none of the *later* graphs shown there represented successful assays and that "especially after this was done, we ran into more severe problems. And it took us a while to do away with the problems;" (4) Ruoslahti also testified that they never determined, in 1979, the affinities of the monoclonal antibodies they used, and that the title of page 43D had been altered at some point—the word "inhibition" had been crossed out and "sandwich" written in; and (5) the testimony of Engvall was that there was nothing about the shape of those curves which indicates that they were sandwich assays. We also note, as evidence bearing upon the credibility of Ruoslahti's testimony (that LJCRF actually reduced the claimed invention to practice in 1979), that when LJCRF attempted to provoke an interference in the PTO with Hybritech based on the U.S. filing of an application that was the counterpart to a Swedish application disclosing similar subject matter, LJCRF could not demonstrate even a *prima facie* reduction to practice prior to Hybritech's August 4, 1980, filing date. During that proceeding, the earliest dates Ruoslahti set down on paper to support conception and reduction to practice were in 1980.

2. *The Work of Oi/Herzenberg Is Not the Claimed Invention*

It is axiomatic that for prior art to anticipate under § 102 it has to meet every element of the claimed invention, and that such a determination is one of fact. *See, e.g., Lindemann*, supra, 730 F.2d at 1458, 221 USPQ at 485; *Great Northern Corp. v. Davis Core & Pad Co.*, 782 F.2d 159, 165, 228 USPQ 356, 358 (Fed.Cir.1986). Section 102(g) upon which the district court relied is one type of "anticipation," i.e., prior invention by another of the same invention. Drs. Oi and Herzenberg testified that their work did not involve detecting the presence of or quantitating antigen but a determination of the number and location of epitopes on a *known* quantity of antigen. Although this work did involve a sandwich assay to the extent that an antigen was sandwiched between two monoclonal antibodies, it is clear that the similarity between that work and the claimed invention goes no further. Furthermore, both doctors testified that they did not know the affinities of the antibodies that were used in their mapping work and in fact never calculated them. Ciotti, Monoclonal's expert, testified that the 10^8 affinity limitation cannot be found anywhere in the Oi/Herzenberg work. Again we are left with a definite and firm conviction that a mistake was made because that work does not meet every element of the claimed invention. The district court's finding to the contrary is clearly erroneous.

We note that the district court, in also holding the patent invalid under § 103, next considered, combined the Oi/Herzenberg work with the Frankel reference, one justifiable inference therefrom being that the court recognized that Frankel discloses a claim *element* that Oi/Herzenberg does not, namely, at least about 10^8 liters/mole affinity.

IV. *Obviousness, 35 U.S.C. § 103*

[6, 7] A section 103 obviousness determination—whether the claimed invention *would have been* (not "would be" as the court repeatedly stated because Monoclonal's pretrial papers used that improper language) obvious at the time the invention was made is reviewed free of the clearly erroneous standard although the underlying factual inquiries—scope and content of the prior art, level of ordinary skill in the art,[3] and differences between the prior art

3. Although the district court failed expressly to find the level of ordinary skill in the art at the

and the claimed invention—integral parts of the subjective determination involved in § 103, are reviewed under that standard. Objective evidence such as commercial success, failure of others, long-felt need, and unexpected results must be considered *before* a conclusion on obviousness is reached and is not merely "icing on the cake," as the district court stated at trial. *See Lindemann*, supra, 730 F.2d at 1461, 221 USPQ at 488; *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 218 USPQ 871 (Fed. Cir.1983); *Kansas Jack, Inc. v. Kuhn*, 719 F.2d 1144, 219 USPQ 856 (Fed.Cir.1983); *W.L. Gore & Associates v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303, 314 (Fed.Cir. 1983).

1. *The Eight Articles "Predicting" Widespread Use of Monoclonal Antibodies*

[8] Before discussing the more pertinent references in this case—the Oi/Herzenberg and Frankel works—we cull the other prior art references relied on by the trial court.

First, the latest four of the eight articles that the court stated were of the "utmost importance" because they "predicted" that the breakthrough in production of monoclonal antibodies by Kohler and Milstein would lead to widespread use of monoclonal antibodies in immunoassays are neither 102(a)/103 nor 102(b)/103 prior art because they are dated between late 1979 and March 6, 1980, well after the date of conception and within one year of the filing date of the '110 patent.

The earliest four of the eight articles, on the other hand, although clearly prior art, discuss *production* of monoclonal antibodies—admittedly old after Kohler and Milstein showed how to produce them—but none discloses sandwich assays. At *most*, these articles are invitations to try monoclonal antibodies in immunoassays but do not suggest how that end might be accomplished. To the extent the district court relied upon these references to establish that it would have been *obvious to try* monoclonal antibodies of 10^8 liters/mole affinity in a sandwich immunoassay that detects the presence of or quantitates antigen, the court was in error. *See Jones v. Hardy*, 727 F.2d 1524, 1530, 220 USPQ 1021, 1026 (Fed.Cir.1984) ("Obvious to try" is improper consideration in adjudicating obviousness issue).[4]

2. *The Kohler and Milstein Work, the Cuello Article and the Jeong, Piasio, and Schurr Patents Considered by the Examiner*

The district court's finding that Kohler and Milstein developed a method for producing monoclonal antibodies in vitro is correct, but that finding proves no more; although it made possible all later work in that it paved the way for a supply of monoclonal antibodies, it indisputably does not suggest using monoclonal antibodies in a sandwich assay in accordance with the invention claimed in the '110 patent.

The Cuello reference discloses monoclonal antibodies but not in a sandwich assay. The competitive assay in Cuello, moreover,

time the invention was made, it did make reference to "[p]eople working in immunology aware of the Kohler and Milstein discovery" which we deem an accurate finding for the purposes of that portion of the *Graham* factual inquiries.

4. Finding 10, which states that the invention was contemporaneously developed and disclosed in at least five publications and patent applications not listed above *and dated well after the filing date of the '110 patent but before its issuance* is irrelevant for purposes of the hypothesis based on the three factual inquiries required by § 103 as interpreted by *Graham v. John Deere*, 383 U.S. 1, 86 S.Ct. 684, 15 L.Ed.2d 545, 148 USPQ 459 (1966) because obviousness must be determined as of the time the invention was made. Additionally, they are of little probative value in this case because they are dated December 1981 at the earliest, more than a year after the August 4, 1980, filing date here and roughly two years after conception occurred. Furthermore, simultaneous development may or may not be indicative of obviousness, the latter being the case here for the above reasons and because the other evidence of nonobviousness is adequate, such occurrences having been provided for in 35 U.S.C. § 135. *Lindemann*, supra, 730 F.2d at 1460–61, 221 USPQ at 487; *Environmental Designs, Ltd. v. Union Oil Co. of California*, 713 F.2d 693, 698 n. 7, 218 USPQ 865, 869 n. 7 (Fed.Cir.1983).

uses only one monoclonal antibody and thus in no way suggests the claimed invention wherein a ternary complex of two monoclonal antibodies and an antigen form a sandwich. Furthermore, the court did not explain how this art, by itself or in combination with any of the other art, suggests the claimed subject matter and thus why that combination would have been obvious. We are of the opinion that it does not.

The district court correctly found that the use of polyclonal antibodies in sandwich assays was well known. The Jeong patent discloses the use of polyclonal antibodies in a simultaneous sandwich assay, with no suggestion that monoclonal antibodies be so used. It is prior art by virtue of § 102(e), application for the patent having been filed September 5, 1978, its effective date as a reference. The Piasio patent, disclosing a reverse sandwich assay using polyclonal antibodies, and Schurrs, disclosing a forward sandwich assay using the same, both § 102(a) prior art, are likewise devoid of any suggestion that monoclonal antibodies can be used in a similar fashion.

3. *The Oi/Herzenberg Work and the Frankel Article*

Clearly, the most pertinent items of prior art not cited by the examiner are the Oi/Herzenberg work, as described in section A, 3, supra, and the Frankel article. As stated in the discussion of Prior Invention of Another (section III, 2, supra), the Oi/Herzenberg work involved mapping epitopes on a known quantity of antigen. It was not concerned with and does not disclose using monoclonal antibodies of at least 10^8 liters/mole affinity. Oi and Herzenberg testified that they did not know the affinity of the antibodies used, and Ciotti testified that nowhere in that work is there mention of monoclonal antibody affinity of at least 10^8 liters/mole. On this basis, we conclude that the Oi/Herzenberg work is qualitatively different than the claimed invention; the former is directed to mapping epitopes on a known quantity of antigen and the latter to determining the "presence or concentration of an antigenic substance in a sample of fluid...." We disagree with Monoclonal that these are "essentially the same thing." Furthermore, it is perfectly clear that this work in no way suggests using monoclonal antibodies of the affinity claimed in the '110 patent. It is because of these differences between the Oi/Herzenberg work and the claimed invention that the fact that an antigen was sandwiched between two monoclonal antibodies in the course of Oi's and Herzenberg's work is not sufficient basis to conclude that the claimed invention would have been obvious at the time it was made to a person of ordinary skill in the art.

Likewise, a conclusion that the invention would have been obvious cannot properly be reached when the Oi/Herzenberg work is considered in view of the Frankel article. Frankel teaches a method for rapid determination of affinity constants for monoclonal antibodies, some of which clearly have affinities of the order defined by the claims, but does not in any way suggest using two of those antibodies in a sandwich to assay an antigen by forming a ternary complex of labelled antibody, the antigenic substance, and a bound antibody wherein the presence of the antigenic substance is determined by measuring either the amount of labelled antibody bound to a solid carrier or the amount of unreacted labelled antibody. The mere existence of prior art disclosing how to measure the affinity of high affinity monoclonal antibodies is insufficient to support a holding of obviousness. Hybritech's claims define a *process* that *employs* monoclonal antibodies, and does not merely claim antibodies of high affinity. In view of the fact that the Oi/Herzenberg work is not directed to an assay as claimed and does not disclose antibodies of at least 10^8 liters/mole affinity, and further that Frankel fails to suggest using such antibodies in a sandwich assay, the Frankel article does not compensate for the substantial difference between the Oi/Herzenberg work and the claimed subject matter, and therefore those references in combination cannot support a holding of obviousness.

4. *Objective Evidence of Nonobviousness*

In one part of its opinion the court found that "the commercial success of the kits *may* well be attributed to the business expertise and acumen of the plaintiff's personnel, together with its capital base and marketing abilities" (emphasis ours) and later that "[w]here commercial success is based on the sudden availability of starting materials, in this instance the availability of monoclonal antibodies as a result of the Kohler and Milstein discovery, business acumen, marketing ability, and capital sources, no causal relationship is proven." (Citation omitted.)

i. *Commercial Success: Hybritech's Diagnostic Kits Grabbed a Substantial Market Share*

The undisputed evidence is that Hybritech's diagnostic kits had a substantial market impact. The first diagnostic kit sales occurring in mid–1981, sales increased seven million dollars in just over one year, from $6.9 million in 1983 to an estimated $14.5 million in 1984; sales in 1980 were nonexistent. Competing with products from industry giants such as Abbott Labs, Hoffman LaRoche, Becton-Dickinson, and Baxter-Travenol, Hybritech's HCG kit became the market leader with roughly twenty-five percent of the market at the expense of market shares of the other companies. Its PAP kit ranks second only to a product sold by Dupont's New England Nuclear, surpassing products from Baxter-Travenol, Abbott, and others. Hybritech's other kits, indisputably embodying the invention claimed in the '110 patent, obtained similar substantial market positions.

Although the district court did not provide its insights into why commercial success was due to business acumen and not to the merits of the claimed invention, Monoclonal urges in support that it was due to Hybritech's spending disproportionate sums on marketing, 25–30% of income. The undisputed evidence was that expenditures of *mature* companies in this field are between 17 and 32%. Furthermore, the record shows that advertising makes those in the industry—hospitals, doctors, and clinical laboratories—aware of the diagnostic kits but does not make these potential users buy them; the products have to work, and there is no evidence that that is not the case here or that the success was not due to the merits of the claimed sandwich assays—clearly contrary to the district court's finding.

The trial court's finding that the "sudden availability of monoclonals" was the reason for the commercial success of Hybritech's diagnostic kits (Finding 11) is unsupported by the record and clearly erroneous. Monoclonal admits that monoclonal antibodies were available in the United States in *1978*, and the evidence clearly reflects that. Thus, at least *three years* passed between the time monoclonal antibodies were available in adequate supply and the time Hybritech began selling its kits. Especially in the fast-moving biotechnology field, as the evidence shows, that is anything but sudden availability.

ii. *Unexpected Advantages*

Hybritech points to the testimony of three witnesses skilled in the diagnostic field who state that, based on tests done in their laboratories as a result of real-world comparisons in the normal course of research, the diagnostic kits that embody the '110 invention unexpectedly solved longstanding problems. Dr. Hussa, the head of a large referral laboratory and a world-wide consultant, testified that until Hybritech introduced its kits, he and others were very skeptical and had almost exclusively used competitive assays with a radioactive tracer (RIAs).[5] In relation to an

5. Monoclonal's expert Blakemore testified that of 425 assays on the market in 1979 less than 1% were sandwich assays. Today, sandwich assays constitute the majority of all assays sold.

The record also shows that Blakemore, who testified extensively for Monoclonal that the claimed invention would have been obvious, never used monoclonal antibodies in sandwich assays at Bio-Rad before 1980. Additionally, she did not even mention them in the Jeong patent, of which she was a coinventor, which

HCG Hybritech kit, he testified that he had first thought that the Hybritech HCG kit would not give accurate results for low antigen concentrations because that condition is indicated in the Hybritech kit by a low radioactivity reading, a reading difficult to differentiate from control samples containing no antigen. He also stated that in the past, RIA kits falsely detected HCG in nonpregnant women, a condition which would indicate cancer and surgery. He stated that when he employed the Hybritech HCG kit in such instances it demonstrated, correctly and absent any difficulty interpreting the data, that no HCG was present.

Dr. Blethen, an M.D. holding a Ph.D. in biochemistry, testified that she did not think that the Hybritech HGH kit, for detecting growth hormone in children, would offer any advantage, but she determined that it detected HGH deficiencies in children where conventional RIAs failed to do so. She also stated that the kit does not give false positive readings as do conventional RIA kits, an opinion shared by Dr. Hussa. A third witness, Dr. Herschman, who holds a master's degree in chemistry, testified that he spent years working on the development of an assay that would determine the presence of TSH (thyroid stimulating hormone) with greater sensitivity. He succeeded but discovered that the Hybritech TSH kit had the same sensitivity, the test being performed in four hours rather than the three days his kit required.

Having considered the evidence of nonobviousness required by § 103 and *Graham*, supra, we hold, as a matter of law, that the claimed subject matter of the '110 patent would not have been obvious to one of ordinary skill in the art at the time the invention was made and therefore reverse the court's judgment to the contrary. The large number of references, as a whole, relied upon by the district court to show obviousness, about twenty in number, skirt all around but do not as a whole suggest the claimed invention, which they must, to overcome the presumed validity, *Lindemann*, 730 F.2d at 1462, 221 USPQ at 488, *as a whole*. *See* 35 U.S.C. § 103; *Jones v. Hardy*, 727 F.2d 1524, 1529, 220 USPQ 1021, 1024 (Fed.Cir.1984). Focusing on the obviousness of substitutions and differences instead of on the invention as a whole, as the district court did in frequently describing the claimed invention as the mere substitution of monoclonal for polyclonal antibodies in a sandwich assay, was a legally improper way to simplify the difficult determination of obviousness. *See generally Hodosh v. Block Drug Co.*, 786 F.2d 1136, 229 USPQ 182 (Fed.Cir.1986).[6]

With respect to the objective indicia of nonobviousness, while there is evidence that marketing and financing played a role in the success of Hybritech's kits, as they do with any product, it is clear to us on the entire record that the commercial success here was due to the merits of the claimed invention. It cannot be argued on this record that Hybritech's success would have been as great and as prolonged as admittedly it has been if that success were not due to the merits of the invention. The evidence is that these kits compete successfully with numerous others for the trust of persons who have to make fast, accurate, and safe diagnoses. This is not the kind of

issued January 13, 1981, long after the beginning of Hybritech's work in this area in 1979.

6. It bears repeating that it is crucial that counsel set forth the law accurately. More particularly, it is the duty of counsel to impart to the judge that the obviousness question properly is whether the *claimed* invention *as a whole would have been* obvious to one of *ordinary* skill in the art *at the time the invention was made*, and that the district court must *expressly* make the three factual determinations required by *Graham* and consider objective evidence of obviousness *before* the legal conclusion of obviousness vel non is made. Submitting to the court language like "any differences ... would have been obvious," as was done here, violates the axiom that the question is not whether the differences would have been obvious but the claimed invention *as a whole*. Furthermore, arguing that "it would be obvious" rather than that it would *have been* obvious shifts the court's focus to the wrong period of time, namely to a time long after the invention was made, in which, more likely than not, the prior art and the level of ordinary skill in the art are more advanced. *See* 35 U.S.C. § 103.

merchandise that can be sold by advertising hyperbole.

V. *Enablement, Best Mode, and Definiteness Under § 112*

The section 112 defense appears to have been an afterthought of both Monoclonal, who briefly but unsuccessfully attempts to defend this utterly baseless determination, and of the district court which adopted the defense from Monoclonal's pretrial papers apparently without knowledge of the applicable law, to highlight, as it stated at trial, that it was part of its job to see that "whoever wins wins all the way or whoever loses loses all the way." Taken as a whole, the court's comments on § 112—split into two parts, one from Monoclonal's pretrial brief and the other from the adopted pretrial findings and conclusions—are internally inconsistent. The opinion states that the patent fails to disclose how (1) to make monoclonal antibodies; (2) to screen for proper monoclonal antibodies; and (3) to measure monoclonal antibody affinity and therefore the specification is nonenabling and does not satisfy the best mode requirement, and the claims are indefinite. We discuss each of these in turn.

1. *Enablement*

[9, 10] Enablement is a legal determination of whether a patent enables one skilled in the art to make and use the claimed invention, *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 960, 220 USPQ 592, 599 (Fed. Cir.1983), is not precluded even if some experimentation is necessary, although the amount of experimentation needed must not be unduly extensive, *Atlas Powder Co. v. E.I. Du Pont De Nemours & Co.*, 750 F.2d 1569, 1576, 224 USPQ 409, 413 (Fed. Cir.1984), and is determined as of the filing date of the patent application, which was August 4, 1980. *See W.L. Gore and Associates v. Garlock, Inc.*, 721 F.2d 1540, 1556, 220 USPQ 303, 315 (Fed.Cir.1983). Furthermore, a patent need not teach, and preferably omits, what is well known in the art. *Lindemann*, 730 F.2d at 1463, 221 USPQ at 489.

[11] The record fully supports the '110 patent's statement that

> The monoclonal antibodies used for the present invention are obtained by the [hybridoma] process discussed by Milstein and Kohler.... The details of this process are well known and not repeated here.

The district court itself stated that the "method for producing monoclonal antibodies in vitro was well known prior to the alleged invention of the '110 patent," and used the "sudden availability of monoclonal antibodies" produced by the Kohler and Milstein discovery to support, albeit erroneously, its finding of a lack of nexus between the merits of the claimed invention and its commercial success. The court then about-faced and held the '110 patent deficient because it fails to teach how to make monoclonal antibodies.

With respect to screening, the only permissible view of the evidence is that screening methods used to identify the necessary characteristics, including affinity, of the monoclonal antibodies used in the invention were known in the art and that the '110 patent contemplated one of those. At trial, Monoclonal's counsel stated "it is a procedure that was known in '78." In similar fashion, the district court held that the claimed subject matter would have been obvious in part because the "existence of monoclonal antibodies *having the affinity constants claimed in the patent was well known* prior to the alleged invention...." [Emphasis ours.] Furthermore, there was not a shred of evidence that undue experimentation was required by those skilled in the art to practice the invention. We hold as a matter of law that the '110 patent disclosure is enabling.

2. *Best Mode*

[12, 13] "The specification ... shall set forth the best mode contemplated by the inventor of carrying out his invention." 35 U.S.C. § 112. Because not complying with the best mode requirement amounts to concealing the preferred mode contemplated by the applicant at the time of filing, in order to find that the best mode requirement is not satisfied, it must be shown that

the applicant knew of and concealed a better mode than he disclosed. *DeGeorge v. Bernier*, 768 F.2d 1318, 1324, 226 USPQ 758, 763 (Fed.Cir.1985) (quoting with approval *In re Sherwood*, 613 F.2d 809, 204 USPQ 537 (CCPA 1980)). The only evidence even colorably relating to concealment is testimony by various Hybritech employees that sophisticated, competent people perform the screening and that the screening process is labor-intensive and time-consuming. It is not plausible that this evidence amounts to proof of concealment of a best mode for screening or producing monoclonal antibodies for use in the claimed '110 process, and therefore we are of the firm conviction that the district court's finding that the best mode requirement was not satisfied is clearly erroneous.

3. *Indefiniteness*

[14] The basis of the district court's holding that the claims are indefinite is that "they do not disclose how infringement may be avoided because antibody affinity cannot be estimated with any consistency." (Conclusion 6.) Even if the district court's finding in support of this holding—that "there is no standard set of experimental conditions which are used to estimate affinities"—is accurate, under the law pertaining to indefiniteness—"if the claims, read in light of the specification, reasonably apprise those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits, the courts can demand no more," *Shatterproof Glass Corp. v. Libbey-Owens Ford Co.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed.Cir.1985)—the claims clearly are definite. The evidence of record indisputably shows that calculating affinity was known in the art at the time of filing, and notwithstanding the fact that those calculations are not precise, or "standard," the claims, read in light of the specification, reasonably apprise those skilled in the art and are as precise as the subject matter permits. As a matter of law, no court can demand more.

VI. *Motions*

Monoclonal's motion to strike Appendices A and B of Hybritech's reply brief as being beyond the page limit applicable to reply briefs is granted as to Appendix A but denied as to Appendix B, the latter having been helpful in culling the often non-supportive citations to the record by Monoclonal.

Hybritech's motion to supplement the record with a Monoclonal advertisement not considered at trial is denied. Any adverse impact that the disposition of these two motions has upon either party is more than outweighed by this court's patience with the seemingly endless flow of post-argument argumentative papers.

VII. *Conclusion*

The judgment of the district court holding the patent in suit invalid is *reversed* in all respects, and the case is *remanded* for a determination of the issue of infringement which the court held was moot.

REVERSED AND REMANDED.

TELLER ENVIRONMENTAL SYSTEMS, INC., Appellant,

v.

UNITED STATES of America, Appellee.

Appeal No. 85-2676.

United States Court of Appeals, Federal Circuit.

Oct. 1, 1986.

Appeal was taken by contractor from a decision of the Armed Services Board of Contract Appeals determining its liability to government for correcting defective repair work. The Court of Appeals, Archer, Circuit Judge, held that decision of Board that there were latent defects in repair

counsel jointly and severally liable for payment to Mor-Flo of the damages we assess.

CONCLUSION

For the foregoing reasons, the judgment appealed from is affirmed. Because State has show no arguable basis in law or fact for reversal, and has argued its appeal with distortion and disregard of the record and the controlling law, we deem the appeal to have been frivolous as filed and frivolous as argued, and grant Mor-Flo's request for sanctions under Fed. R. App. P. 38. Therefore, Mor-Flo is awarded the sum of $5,000 as damages for defending this frivolous appeal, for the payment of which State and its counsel are jointly and severally liable.

AFFIRMED — SANCTIONS IMPOSED.

Court of Appeals, Federal Circuit

Continental Can Co. USA Inc. v. Monsanto Co.

No. 90-1328

Decided November 13, 1991

JUDICIAL PRACTICE AND PROCEDURE

1. Procedure — Summary judgment — Patents (§410.3303)

Summary judgment is as available in patent cases as in other areas of litigation and can facilitate disposition of legally meritless suits, but improvident grant of summary judgment can prolong litigation and increase its burdens, especially in patent disputes in which patent property is wasting asset.

PATENTS

2. Patentability/Validity — Anticipation — In general (§115.0701)

Anticipation under 35 USC 102 cannot be found if more than one reference is required to establish unpatentability of claimed invention; rather, validity in such case is determined pursuant to 35 USC 103.

3. Patentability/Validity — Anticipation — Prior art (§115.0703)

Patent construction — Claims — Defining terms (§125.1305)

Federal district court erred by ruling, on summary judgment, that claims for patented bottle were anticipated by prior art, since court erred in its construction of claim term "hollow," and since disputed issue of fact exists as to whether injection blow molding process necessarily produced "hollow" ribs in prior art base structure, as term "hollow" is used in patent.

4. Patentability/Validity — Anticipation — Prior sale — In general (§115.0707.01)

"On sale" bar of 35 USC 102(b) does not arise simply because intended customer was participating in development and testing, but rather all circumstances concerning relationship between patentee and customer must be considered in light of public policy underlying Section 102(b); thus, federal district court erred in determining that bottle was "on sale," in view of evidence showing that bottle was part of terminated development project that never bore commercial fruit and was cloaked in confidentiality.

5. Patentability/Validity — Obviousness — Combining references (§115.0905)

Federal district court erred by ruling, on summary judgment, that claimed bottom structure for plastic container was obvious, since, drawing all reasonable inferences in favor of patentee, it has not been established that person skilled in art would be motivated to select and combine features from each prior art source to make patented base.

6. Patentability/Validity — Obviousness — Secondary considerations generally (§115.0907)

Differences between patented invention and prior art which may appear technologically minor nonetheless can have practical impact, particularly in crowded field, and in such case objective indicia, such as commercial success, or filling existing need, illuminate technological and commercial environment of inventor, and aid in understanding state of art at time invention was made.

7. Patentability/Validity — Obviousness — Commercial success (§115.0908)

Patented invention need not be solely responsible for commercial success in order for this factor to be given appropriate weight.

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the brief."); *Browning Debenture Holders' Comm. v. DASA Corp.*, 605 F.2d 35, 41 (2d Cir. 1978); *Hilmon*, 899 F.2d at 254 (collecting cases); Coghlan, 852 F.2d at 817-18; *TIF Instr. Inc. v. Colette*, 713 F.2d 197, 201 (6th Cir. 1983) (Nies, J., sitting by designation); *Hatch v. Reliance Ins. Co.*, 758 F.2d 409, 416 (9th Cir.), *cert. denied*, 474 U.S. 1021 (1985); *Braley*, 832 F.2d at 1511 (collecting cases); *Saltany v. Reagan*, 886 F.2d 438, 441 (D.C. Cir. 1989), *cert. denied*, 110 S.Ct. 2172 (1990).

Particular patents — General and mechanical — Plastic bottle

4,108,324, Krishnajumar, Roy, Pocock, Das, and Mahajan, ribbed beverage bottle structure for plastic container created by plastic hot-fill, summary judgment of invalidity vacated in part, reversed in part, and remanded.

Appeal from the U.S. District Court for the Southern District of Ohio, Spiegel, J.; 11 USPQ2d 1761.

Patent infringement action brought by Continental Can Co. USA Inc. and Continental Pet Technologies Inc. against Monsanto Co., Hoover Universal Inc., and Johnson Controls Inc. From federal district court decision entering summary judgment in favor of defendants, plaintiffs appeal. Vacated in part, reversed in part, and remanded.

Eugene F. Friedman, Chicago, Ill. (Edwin C. Thomas, III and David M. Novak, of Bell, Boyd & Lloyd, Chicago; Kurt L. Grossman, of Wood, Herron & Evans, Cincinnati, Ohio, with him on brief), for plaintiff-appellants.

Henry J. Renk, New York, N.Y. (Lawrence F. Scinto and Bruce C. Haas, of Fitzpatrick, Cella, Harper & Scinto, New York; Jacob K. Stein and Deborah DeLong, of Thompson, Hine & Flory, Cincinnati, Ohio; Lawrence L. Limpus, St. Louis, Mo., and Edward L. Levine, Milwaukee, Wis., with him on brief), for defendants-appellees.

Before Newman, Archer, and Rader, circuit judges.

Newman, J.

Continental Can Company USA and Continental PET Technologies (collectively "Continental") appeal the partial summary judgment of the United States District Court for the Southern District of Ohio, holding that United States Patent No. 4,108,324 (the Conobase or '324 patent) is invalid.[1] Final judgment was entered on this issue, for the purpose of appeal.

Summary Judgment

An issue may be decided on motion for summary judgment when there is no genuine issue of material fact, and the movant is entitled to judgment as a matter of law. Fed. R. Civ. P. 56(c); *Anderson v. Liberty Lobby, Inc.*, 477 U.S. 242 (1986); *Celotex Corp. v. Catrett*, 477 U.S. 317, 325-26 (1986); *Scripps Clinic & Research Foundation v. Genentech, Inc.*, 927 F.2d 1565, 1571, 18 USPQ2d 1001, 1005 (Fed. Cir. 1991). The movant's burden is to show that no fact material to the issue is in dispute, that even if all material factual inferences are drawn in favor of the non-movant the movant is entitled to judgment as a matter of law. *Id.* Summary judgment is as available in patent cases as in other areas of litigation. *Chore-Time Equipment, Inc. v. Cumberland Corp.*, 713 F.2d 774, 778-79, 218 USPQ 673, 675 (Fed. Cir. 1983)

The purpose of the summary process is to avoid a clearly unnecessary trial, *Matsushita Elec. Industrial Co. v. Zenith Radio Corp.*, 475 U.S. 574, 587 (1986); it is not designed to substitute lawyers' advocacy for evidence, or affidavits for examination before the fact-finder, when there is a genuine issue for trial. As stated in *Adickes v. S.H. Kress & Co.*, 398 U.S. 144, 176 (1970) (Black, J., concurring), "[t]he right to confront, cross-examine and impeach adverse witnesses is one of the most fundamental rights sought to be preserved by the Seventh Amendment". *See also Poller v. Columbia Broadcasting System, Inc.*, 368 U.S. 464, 473 (1962).

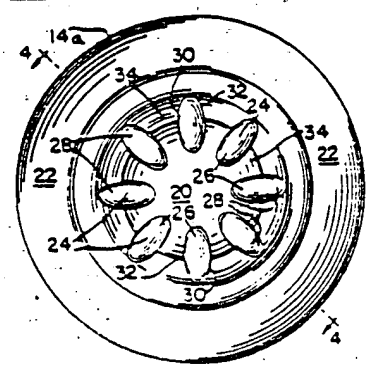
[1] While facilitating the disposition of legally meritless suits, when summary judgment is improvidently granted the effect is to prolong litigation and increase its burdens. This is of particular concern in patent disputes, where the patent property is a wasting asset, and justice is ill served by delay in final resolution. In the case at bar, although some issues could be resolved on the law and undisputed facts, other issues require trial.

The Patented Invention

The '324 patent, entitled "Ribbed Bottom Structure for Plastic Container", inventors Suppayan M. Krishnakumar, Siegfried S. Roy, John F. E. Pocock, Salil K. Das, and Gautam K. Mahajan, is directed to a plastic bottle whose bottom structure has sufficient flexibility to impart improved impact resistance, combined with sufficient rigidity to resist deformation under internal pressure. The patented bottle is said to provide a superior combination of these properties. The bottom structure is illustrated as follows:

[1] *Continental Can Co. USA v. Monsanto Co.*, 11 USPQ2d 1761 (S.D. Ohio 1989), *reconsid. denied*, No. C-1-86-1213 (S.D. Ohio Nov. 9, 1989).

FIG. 2



Claim 1 is the broadest claim of the '324 patent:

1. A container having a sidewall and a bottom structure closing the container at an end portion of the sidewall,

the outer surface of the bottom structure comprising a central concavity,

a convex heel surrounding the concavity and merging therewith and with the sidewall end portion, the lowermost points of the heel lying in a common plane,

and a plurality of ribs interrupting the outer surface of the concavity and distributed in a symmetrical array,

each rib extending longitudinally in the direction of the heel and downwardly from an inner portion of the concavity, whereby the outer end portion of each rib is lower than the inner end portion thereof,

characterized by the feature that the ribs are hollow.

Claims 2 through 5 include additional limitations, described as contributing to the structure's rigidity, flexibility, or both. Claim 2 specifies the ratios of thickness of the walls of the bottom structure to the thickness of the sidewall end portions. Claims 3 specifies that the margins of each rib merge smoothly with adjacent portions of the bottom structure. Claim 4 specifies that each rib is convex relative to the bottom structure. Claim 5 specifies that each rib is of fusiform (a gently tapered shape at the ends) configuration. Each claim carries an independent presumption of validity, 35 U.S.C. § 282, and stands or falls independent of the other claims. *Altoona Publix Theatres, Inc. v. American Tri-Ergon Corp.*, 294 U.S. 477, 487 [24 USPQ 308] (1935).

Continental brought suit for patent infringement against Monsanto Company and Monsanto's successor in this business, Hoover Universal, Inc. and Hoover's parent company, Johnson Controls (collectively "Monsanto"). Monsanto moved for partial summary judgment based on issues of validity under 35 U.S.C. §§ 102 and 103.

I

35 U.S.C. § 102(a)

The statutory requirement that a patented invention be "new" is tested in accordance with 35 U.S.C. § 102(a), which provides that:

§ 102. A person shall be entitled to a patent unless—

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent. . . .

The district court found that all the claims of the '324 patent were anticipated by U.S. Patent No. 3,468,443 (the Marcus patent). We conclude that the district court erred in claim interpretation, and also found disputed facts adversely to the nonmovant, thus inappropriately deciding the issue summarily.

[2] Anticipation under § 102(a) requires that the identical invention that is claimed was previously known to others and thus is not new. *Scripps Clinic*, 927 F.2d at 1576, 18 USPQ2d at 1010; *Titanium Metals Corp. of Am. v. Banner*, 778 F.2d 775, 780, 227 USPQ 773, 777-78 (Fed. Cir. 1985); *Lindemann Maschinenfabrik GmbH v. American Hoist and Derrick Co.*, 730 F.2d 1452, 1458, 221 USPQ 481, 485 (Fed. Cir. 1984). When more than one reference is required to establish unpatentability of the claimed invention anticipation under § 102 can not be found, and validity is determined in terms of § 103.

It was Monsanto's burden to show that every element of the several claims of the '324 patent was identically described in the asserted anticipating reference, the Marcus patent. The district court focused on the term "characterized by the feature that the ribs are hollow", which limits all of the '324 patent claims. Continental argues that the district court incorrectly construed this term, as a matter of law, and that the Marcus patent shows ribs that are not hollow, as that term is used in the '324 patent. Continental also points to other differences between the '324 claims and the description in the Marcus patent.

The Marcus patent rib structure is illustrated in Figure 5 and in cross-section in Figure 6:

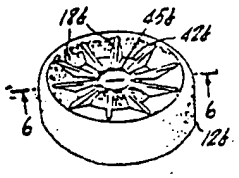


FIG. 5

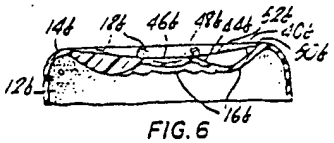


FIG. 6

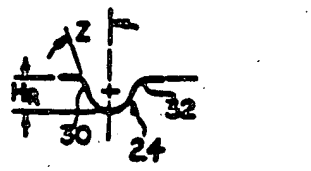
The Marcus patent does not state that its ribs are "hollow", or use a similar term. Continental's witnesses testified by deposition that the Marcus patent shows solid, not hollow, ribs. A witness (Adomaitis) had stated in an internal memorandum written at Continental in 1969, well before this litigation arose, that "the ribs of their [Marcus'] web can be made of solid beams only." Another witness, '324 co-inventor Pocock, testified that:

> It seems evident to me that he [Marcus] was trying to produce some kind of container integrity by the production of essentially solid ribs on the bottom of the bottle. It seems to go to great length here to illustrate them as such.

Krishnakumar, another co-inventor, testified that it "is very obvious the ribs are shown solid", and that Figures 5 and 6 as well as Figures 7 through 12 of the Marcus patent all show solid ribs. However, Marcus, testifying for Monsanto, testified that his ribs were hollow, and that conventional blow molding would inherently produce hollow ribs.

The district court defined "hollow" as meaning that "the inside contour of the ribs generally follows the outside contour thereof", a definition on which the parties agreed. *Continental*, 11 USPQ2d at 1764. See the court's opinion, 11 USPQ2d at 1764-68, for various sketches made by the witnesses. Continental states that the district court erred in construing "hollow", and that the phrase "characterized by the feature that the ribs are hollow" must be construed in terms of the patent in which it appears. *See, e.g., Tandon Corp. v. United States Int'l Trade Comm'n*, 831 F.2d 1017, 1021, 4 USPQ2d 1283, 1286 (Fed. Cir. 1987). The '324 patent explicitly distinguished the Marcus teachings, stating that the '324 ribs are, unlike Marcus, not filled with plastic. The '324 specification uses the term "hollow", as do the prosecution history and the claims, for this purpose The '324 patent's usage of "hollow" is illustrated in rib cross-section in Figure 5A:

FIG. 5A



The Marcus patent's rib structure thus was explicitly differentiated by the term "hollow" as used in the '324 specification, drawings, and prosecution history. Since the claim term must be construed as used by the patentee, the district court erred in its construction of the '324 claim term "hollow". On correct claim construction, the factual question of anticipation must be decided.

Monsanto's argument is that hollow ribs were inherently produced by Marcus. Monsanto thus argues that anticipation lies because the Marcus patent's ribs are "inherently" hollow, regardless of how they are shown in the Marcus patent. Monsanto argues that because the Marcus ribs are formed by injection blow molding, which is the same process described for the Conobase '324 ribs, hollow ribs are inherently disclosed in the Marcus patent.

To serve as an anticipation when the reference is silent about the asserted inherent characteristic, such gap in the reference may be filled with recourse to extrinsic evidence. Such evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. *In re Oelrich*, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981) (quoting *Hansgirg v. Kemmer*, 102 F.2d 212, 214, 40 USPQ 665, 667 (CCPA 1939)) provides:

> Inherency, however may not be established by probabilities or possibilities. The mere fact that a certain thing *may* result from a given set of circumstances is not sufficient. [Citations omitted.] If, however, the disclosure is sufficient to show that the natural result flowing from the operation as taught would result in the performance of the questioned function, it seems to be well settled that the disclosure should be regarded as sufficient.

This modest flexibility in the rule that "anticipation" requires that every element of the claims appear in a single reference accommodates situations where the common knowledge of technologists is not recorded in the reference, that is, where technological

facts are known to those in the field of the invention, albeit not known to judges. It is not, however, a substitute for determination of patentability in terms of § 103.

[3] Continental does not dispute the applicability of the injection blow molding process. However, Continental disputes the material of fact of whether this process necessarily produced "hollow" ribs in the Marcus base structure, as the term "hollow" is used in the '324 patent. Resolution of this disputed fact adversely to Continental was improper on summary judgment. The grant of summary judgment of anticipation under § 102(a) is vacated. The issue requires trial.

II

35 U.S.C. § 102(b)

The district court also held that the Marcus bottle was on sale, 35 U.S.C. § 102(b). Section 102(b) bars entitlement to a patent when:

> (b) the invention was . . . in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States. . . .

The Marcus bottle was developed some ten years before the filing date of the '324 patent, during a project wherein Marcus' employer, Admiral Plastics or APL Corporation, entered into agreements with the Coca-Cola Company for the development of a suitable plastic bottle. The agreements provided that Admiral Plastics would make and Coca-Cola would test the bottles, and that if a satisfactory bottle was developed it would be manufactured by Admiral and purchased by Coca-Cola. Minimum commercial quantities and maximum commercial prices were stated in an agreement, and costs were a matter of discussion. Admiral produced a variety of bottle shapes, including the Marcus bottle. The project was terminated after about two years, because the "mechanical performance" requirements were not met as Coca-Cola wrote at the time.

[4] The district court reasoned that this project "called for the eventual marketing of the Marcus bottles once all technical difficulties were resolved", *Continental*, 11 USPQ2d at 1766, and on this basis held that the Marcus bottles were on sale. This holding was in error, for the "on sale" bar of § 102(b) does not arise simply because the intended customer was participating in development and testing. *See Great Northern Corp. v. Davis Core & Pad Co.*, 782 F.2d 159, 164-65, 228 USPQ 356, 358 (Fed. Cir. 1986). In *Baker Oil Tools, Inc. v. Geo Vann, Inc.*, 828 F.2d 1558, 1563-65, 4 USPQ2d 1210, 1213-15 (Fed. Cir. 1987), this court summarized various factors pertinent to the "on sale" bar when there is an issue concerning the relationship between the patentee and the customer: for example, whether there was a need for testing by other than the patentee; the amount of control exercised; the stage of development of the invention; whether payments were made and the basis thereof; whether confidentiality was required; and whether technological changes were made. All of the circumstances attending the relationship must be considered in light of the public policy underlying § 102(b). *UMC Electronics Co. v. United States*, 816 F.2d 647, 656, 2 USPQ2d 1465, 1471-72 (Fed. Cir. 1987), *cert. denied*, 484 U.S. 1025 (1988).

The district court acknowledged that all technical difficulties were not resolved and that no sales were ever made. Although Admiral Plastics' hope was surely commercial sales, and the record shows that prices and quantities were discussed, this does not of itself place the subject matter "on sale" in the sense of § 102(b). The Marcus bottle was part of a terminated development project that never bore commercial fruit and was cloaked in confidentiality. While the line is not always bright between development and being on sale, see generally *UMC Electronics, supra*, in this case the line was not crossed. The "on sale" bar is measured by "the time the public came into possession of the invention", *id.* at 655, 2 USPQ2d at 1471 (quoting *In re Foster*, 343 F.2d 980, 987-88, 145 USPQ 166, 173 (CCPA 1965), *cert. denied*, 383 U.S. 966 [149 USPQ 906] (1966) ("What starts the period running is clearly the availability of the invention *to the public* through the categories of disclosure enumerated in 102(b). . . ." (emphasis in original))). We conclude that the district court erred in holding that the circumstances that here existed placed the Marcus bottles "on sale" in terms of § 102(b). We therefore reverse and direct that on remand judgment on this issue shall be entered in favor of Continental, as a matter of law.

III

35 U.S.C. § 103

Obviousness, 35 U.S.C. § 103, is reviewed as a legal conclusion based upon underlying facts of four general categories, *viz.* the scope and content of the prior art, the differences between the prior art and the claimed invention, the level of ordinary skill at the time the invention was made, and any objective considerations that may be present. *Gra-*

ham v. John Deere Co., 383 U.S. 1, 17 [148 USPQ 459] (1966); *Interconnect Planning Corp. v. Feil*, 774 F.2d 1132, 1137-38, 227 USPQ 543, 547 (Fed. Cir. 1985).

[5] The parties agreed that the scope and content of the prior art was adequately represented by four references: the Marcus patent discussed in Part I *ante*, a patent to Colombo (U.S. Patent No. 3,403,804), and two patents owned by Continental, U.S. Patent No. 3,598,270 (the Petaloid patent), and No. 3,935,955 (the Decaloid patent). They agreed on little else. In granting summary judgment of invalidity for obviousness, the district court found certain disputed material facts and misapplied certain precepts of law. We conclude that the issue was not amenable to summary resolution. Although it is not entirely clear how the references were combined by the court, we shall review the references briefly, in order to explain our conclusion.

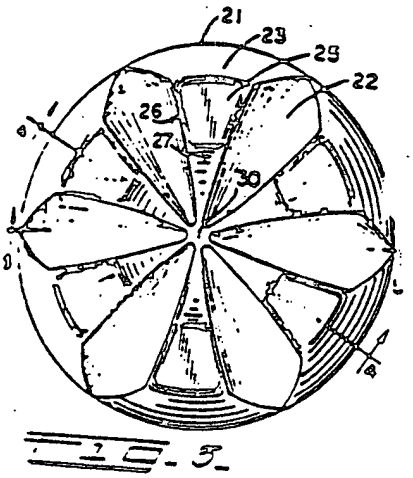


FIG. 5

The Petaloid Patent

The district court referred to the deposition testimony of Siegfried Roy, one of the co-inventors of the '324 patent, that the Petaloid base, inverted, was similar to the Conobase. Continental points out that neither Roy nor any other deponent suggested that the Petaloid base could be or should be inverted, or that inversion would provide an improved base structure. In *In re Gordon*, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984) this court held that although a prior art device could have been turned upside down, that did not make the modification obvious unless the prior art fairly suggested the desirability of turning the device upside down.

Continental points out that the Petaloid description differs in several other ways from the '324 invention. In the '324 structure the outer end of each rib is lower than the inner end, whereas in the Petaloid structure the outer ends of the ribs are higher than the inner ends; that is, the ribs in the Petaloid base extend upward from the center to the sidewall. The Petaloid bottle is supported on feet extending between the ribs, such feet being the locations for stress concentrations. The following drawing is from the Petaloid patent:

Continental states that the '324 Conobase is not only different, but avoids the stress concentrations of the Petaloid device, thus enhancing impact resistance. Monsanto argues that Continental simply used the Petaloid hollow ribs in combination with the Marcus patent. This requires determination of whether there was something in the prior art as a whole to suggest the desirability, and thus the obviousness, of making the combination, in a way that would produce the '324 structure. *See, e.g., Uniroyal, Inc. v. Rudkin-Wiley Corp.*, 837 F.2d 1044, 1051, 5 USPQ2d 1434, 1438 (Fed. Cir.), *cert. denied*, 488 U.S. 825 (1988). Continental argues that it is not apparent, even with hindsight, how any combination of the Petaloid and Marcus patents or other references lead to the '324 base. The Petaloid patent shows concave ribs that extend all the way to the sidewall, while the Marcus ribs extend "from the heel" toward an annular central ring. The Petaloid base has wide, petal-like, open ribs, while Marcus shows narrow, beam-like ribs. The deposition testimony was in conflict as to the inferences drawn from the references.

On this disputed issue, drawing reasonable inferences in favor of the non-movant, it has not been established that one skilled in the art would be motivated to select and combine features from each source in order to make the '324 base. *Interconnect Planning*, 774 F.2d at 1143, 227 USPQ at 551 ("When prior art references require selective combination by the court to render obvious a subsequent invention, there must be some reason for the combination other than the hindsight gleaned from the invention itself").

The Decaloid Patent

The district court also referred to combination of the Decaloid base with the Marcus base. The Decaloid base has ten hollow ribs that extend to the sidewall, and ten feet between the ribs:

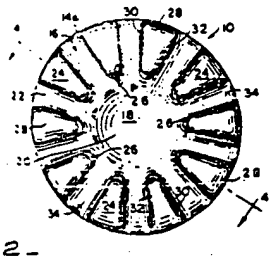
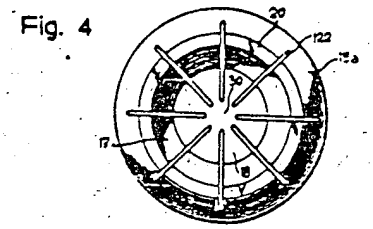


FIG-2-

Monsanto does not explain, and we can not discern, how the combination with Marcus would have led a person of ordinary skill to the '324 base. The court's summary holding of obviousness based on these references, separately or in combination, can not be sustained.

The Colombo Patent

The Colombo base, like the Petaloid and Decaloid bases, has hollow ribs that extend to the sidewall, in a still different structure from that of Marcus and also from that of the '324 patent. Colombo describes his ribs as inverted U-shapes, concave, located on the outer surface of the central concavity:



Again, drawing reasonable factual inferences in favor of Continental, and in the absence of any suggestion or motivation in the prior art as a whole to make a selective combination of the Colombo and Marcus structures along with other changes needed to obtain the '324 structure, summary judgment of obviousness was inappropriate.

The district court found that there was no substantial difference between the '324 invention and the combined teachings of the prior art:

> As obviousness can be established on the basis of the combined teachings of references, we think it is clear that simple enhancements of existing prior art, i.e., inverting the '270 petaloid base, do not constitute a substantial difference between the subject matter claimed in the '324 patent and that of the prior art. Thus, the facts of this case reveal no substantial difference between '324 and the prior art.

Continental, 11 USPQ2d at 1769 (citation omitted). However, as we have discussed, the criterion of § 103 is not whether the differences from the prior art are "simple enhancements", but whether it would have been obvious to make the claimed structure.

Objective Indicia

The district court concluded that the structure in suit is simply a variation on known themes. It is in such circumstance that the objective indicia — the so-called secondary considerations — are most useful to the decision-maker. The significance of a new structure is often better measured in the marketplace than in the courtroom.

[6] Thus when differences that may appear technologically minor nonetheless have a practical impact, particularly in a crowded field, the decision-maker must consider the obviousness of the new structure in this light. Such objective indicia as commercial success, or filling an existing need, illuminate the technological and commercial environment of the inventor, and aid in understanding the state of the art at the time the invention was made. *See In re Piasecki*, 745 F.2d 1468, 1475, 223 USPQ 785, 790 (Fed. Cir. 1984) (secondary considerations "often establish that an invention appearing to have been obvious in light of the prior art was not" (quoting *Stratoflex, Inc. v. Aeroquip Corp.*, 713 F.2d 1530, 1538-39, 218 USPQ 871, 879 (Fed. Cir. 1983))).

Continental licensed the '324 counterpart Japanese patent to a Japanese company, Yoshino, that we are told had been unable to develop a plastic bottle for hot-fill applications. A witness for Toyo Seikan, another Japanese licensee, testified that the Conobase "sustains itself in higher temperatures, and it does not cause buckling after you fill [the bottle]", as compared with previously available plastic bottles. Continental asserts that Monsanto had been unable to develop a satisfactory bottle for hot-fill applications, and had therefore obtained this technology from Yoshino.

[7] The district court acknowledged the commercial success of the Conobase, but stated that "we are not convinced that the conobase *alone* accounts for any of the success." 11 USPQ2d at 1770 (emphasis in original). The court suggested that the commercial success in Japan was due to the market strength of the Japanese licensees, and held that there is no nexus between the merits of the product and its commercial success. It is not necessary, however, that the patented invention be solely responsible for the commercial success, in order for this

factor to be given weight appropriate to the evidence, along with other pertinent factors. *See generally Demaco Corp. v. F. Von Langsdorff Licensing Ltd.*, 851 F.2d 1387, 1392-94, 7 USPQ2d 1222, 1226-28 (Fed. Cir.), *cert. denied*, 488 U.S. 956 (1988); *Rosemount, Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1546, 221 USPQ 1, 7 (Fed. Cir. 1984). Monsanto also states that the Conobase is different from the '324 invention, so that even were the Conobase successful, this does not inure to the benefit of the '324 patent. It is apparent that the factual issues surrounding the objective indicia were disputed, and material.

In view of the material facts requiring resolution, the issue of obviousness was not properly decided on motion for summary judgment. We vacate the grant based on 35 U.S.C. § 103, and remand for trial of this issue and the other issues remaining in the case.

Costs

Costs in favor of Continental.

REVERSED IN PART, VACATED IN PART, and REMANDED

U.S. Patent and Trademark Office
Trademark Trial and Appeal Board

In re Pennzoil Products Co.

Serial No. 73/670,049

Decided August 21, 1991
Released October 10, 1991

TRADEMARKS AND UNFAIR TRADE PRACTICES

1. Types of marks — Descriptive — In general (§327.0301)

Term is considered to be merely descriptive of goods or services, within meaning of Trademark Act's Section 2(e)(1), if it immediately describes ingredient, quality, characteristic, or feature or if it directly conveys information regarding nature, function, purpose, or use of such goods or services; term need not describe all properties or functions of goods or services, but rather is merely descriptive if it describes significant attribute of goods or services.

2. Types of marks — Descriptive — Particular marks (§327.0303)

"MULTI-VIS" is merely descriptive of multiple viscosity motor oil, regardless of whether applicant may have been first to use such term, and/or is currently only entity using term.

3. Types of marks — Generic — Particular marks (§327.0603)

"MULTI-VIS," as applied to multiple viscosity motor oil, is generic and incapable of registration, despite form declarations from nine marketers of automotive oil products as to significance of term; applicant's extensive promotional expenditures, unit sales, product revenues, and position of sales leadership in motor oil are insufficient to demonstrate that "MULTI-VIS" is either promoted as mark or perceived as one by purchasing public.

Appeal from refusal of registration (Fred Mandir, examining attorney; Thomas Howell, managing attorney).

Application for registration of trademark of Pennzoil Products Co., serial no. 73/670,049, filed July 6, 1987. From refusal of registration, applicant appeals. Affirmed.

Frederick B. Ziesenheim, of Webb, Burden, Ziesenheim & Webb, Pittsburgh, Pa., for applicant.

Before Cissel, Quinn, and Hohein, members.

Hohein, member.

An application has been filed by Pennzoil Products Company to register "MULTI-VIS" for "multiple viscosity motor oil".[1]

Registration has been finally refused under Section 2(e)(1) of the Trademark Act, 15 U.S.C. §1052(e)(1), on the basis that, when used in connection with applicant's goods, the mark is merely descriptive of them. Although applicant, in response, amended the application to seek registration pursuant to the provisions of Section 2(f) of the Trademark Act, 15 U.S.C. §1052(f), registration thereunder has also been finally refused on the ground that the evidence of acquired distinctiveness is insufficient since the term "MULTI-VIS" is generic or so inherently and directly descriptive of applicant's goods as to be incapable of registration.[2]

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[1] Ser. No. 73/670,049, filed on July 6, 1987, which alleges dates of first use of April 28, 1953.

[2] Such refusal was expressed in the Office Action of April 4, 1990 as follows:

> The refusal of registration on the ground that the evidence presented fails to demonstrate de jure secondary meaning under Section 2(f) is proper. The proposed mark MULTI-VIS is generic or so highly descriptive as to be incapable of registration.

Brief Definitive Report

THE IMMUNOGENICITY OF CHIMERIC ANTIBODIES

By MARIANNE BRÜGGEMANN,* GREG WINTER,‡ HERMAN WALDMANN,* AND MICHAEL S. NEUBERGER‡

*From the *Division of Immunology, Department of Pathology, Addenbrooke's Hospital, Cambridge CB2 2QQ; and the ‡Medical Research Council Laboratory of Molecular Biology, Cambridge CB2 2QH, United Kingdom*

Owing to problems in making high affinity human mAbs, there is interest in the therapeutic application of chimeric antibodies in which either the V domains or just the hypervariable regions of rodent mAbs have been used to replace the equivalent parts of a human antibody (1, 2, and references therein). Whereas xenogeneic antibodies are highly immunogenic in man (see reference 3 for references), little is known about the immunogenicity of chimeric antibodies. It is unclear to what extent a particular V domain is characteristic of the species from which it originates, and therefore, whether a response will be elicited by an antibody in which only the V region is foreign. If there is such an antiidiotypic response, to what extent is it enhanced by linkage to foreign C domains? Here, we describe experiments carried out in the mouse that address these questions.

Materials and Methods

Mice and Immunizations. Mice were from Olac, Bicester, UK, or National Institute for Medical Research, Mill Hill, UK. Prebleed sera were taken from 6–8-wk-old females (six per group), which were then injected intraperitoneally with the relevant antibody (40 μg) in CFA. Serum was taken 30 d later, and the animals were boosted intraperitoneally with the same antibody (40 μg) in IFA; serum was taken after a further 10 d. For injection with cell-bound antibody, spleen cells from F_1 mice were conjugated with 4-hydroxy-3-nitrophenacetyl (NP)-kephalin (4); mice were immunized intravenously with 5×10^6 syngeneic NP-spleen cells mixed with 40 μg of anti-NP antibody. The boost (day 30) was the same as the primary immunization.

Antibodies and Immunoassays. Antibodies were purified by affinity chromatography (4) from the supernatants of cells of the J558L plasmacytoma (which secretes λ L chains) transfected with plasmids directing the synthesis of the appropriate antibody H chain. The H chain genes for HuV_{NP}-Huγ2, HuV_{NP}-Moγ2b^b, and MoV_{NP}-Moγ2b^b were assembled by inserting C_H exon fragments (7.2-kb Hind III–Bam HI fragment for human γ2 [described in reference 4]; 4.2-kb Eco RI–Bgl II fragment for mouse γ2b^b [ref. 5]) into the pSV-V_{NP} vector or a derivative containing the HuV_{NP} V domain (2). Other transfectants have been described (1, 2, 4).

Antibody responses were measured by ELISA. Serum dilutions were incubated in microtitre plates coated with the relevant IgH, λ anti-NP antibody. Bound antiantibodies were detected using biotinylated anti-mouse κ antiserum and horseradish peroxidase coupled to streptavidin. Immune sera had less than threefold the prebleed titre of residual λ-bearing anti-NP anti-

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body, as well as of antibodies reacting with either mouse IgM, λ myeloma protein, or purified λ L chains.

Results and Discussion

The response was compared of mice injected with one of three antibodies. The most xenogeneic antibody (HuV_{NP}-Huγ2) is composed of a human γ2 C region linked to a V domain that has the framework residues of the human NEW myeloma protein (Fig. 1). A chimeric derivative (in which only the V region frameworks are human) was created by substituting the human Cγ2 by the Cγ2b of C57BL/6 mice to yield HuV_{NP}-Moγ2b^b. In MoV_{NP}-Moγ2b^b (the syngeneic antibody), the entire V domain is of mouse origin, the foreign framework residues having been substituted by mouse sequences. All the antibodies contain a mouse λ L chain, as well as V_H hypervariable region sequences derived from a mouse antibody specific for NP.

Groups of (C57BL/6 × BALB/c)F_1 mice were immunized intraperitoneally with the three antibodies in CFA. The mice made a strong primary and secondary response to the most xenogeneic antibody, a reduced yet nevertheless considerable response to the chimeric antibody, but no detectable response to the syngeneic antibody (Fig. 2 *A*). In the mice immunized with the HuV_{NP}-Huγ2, a large proportion of the response was directed against the human γ2 C region, as witnessed by binding inhibition assays using a human IgG2 myeloma protein; much less inhibition was given by an antibody (HuV_{NP}-Huε) whose H chain is composed of the HuV_{NP} V_H domain linked to human Cε (Fig. 3 *A*, *I*). The anti-V region response elicited by the xenogeneic antibody HuV_{NP}-Huγ2 was measured using a HuV_{NP}-Huε coat; it was of a similar order to that elicited by HuV_{NP}-Moγ2b^b (Fig. 3 *B*). Thus, a considerable proportion of the response to the xenogeneic antibody was directed against the V region; this antiidiotypic response was not diminished by using the chimeric antibody with self C regions.

The antiidiotypic response in the mice immunized with either HuV_{NP}-Huγ2 or HuV_{NP}-Moγ2b^b was not exclusively directed against the human frameworks of the immunizing antibody, although these are the only foreign determinants in the V domain. The mice contained a significant titre of antibodies that recognized MoV_{NP} (Fig. 3, *A* and *B*). A more direct demonstration that it is possible to elicit an antibody response to syngeneic V domains is provided by immunizing mice with MoV_{NP}-Huγ2 (Fig. 3 *B*). Thus, the mouse can make a response to its own V domains, and probably to the hypervariable regions themselves. However, this response is not elicited unless the administered antibody contains some foreign determinants.

As a better system to mimic the use of mAbs directed against tumor cell surface

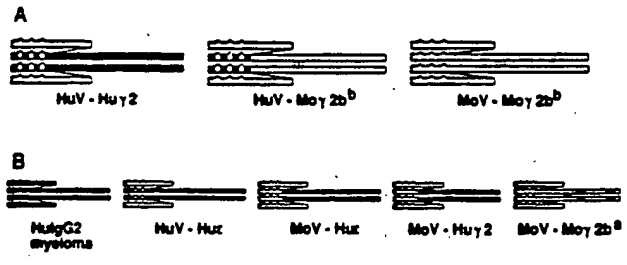
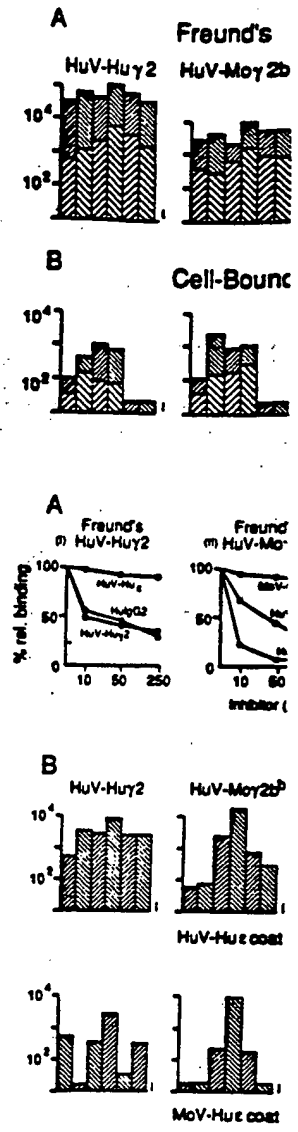


FIGURE 1. Structure of antibodies. (*A*) Antibodies used for immunization. (*B*) Antibodies used for testing the specificity of the responses. The open and filled bars denote sequences of mouse and human origin, respectively. (×) Amino acid positions at which MoV_{NP}-Moγ2b^a and MoV_{NP}-Moγ2b^b differ.



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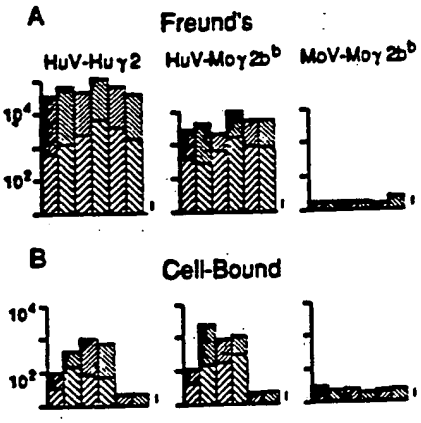

FIGURE 2. Responses to administered antibodies. (*A*) Responses to antibodies emulsified in Freund's. (*B*) Responses to antibodies bound to syngeneic spleen cells. Bars in the histogram give the serum dilution from individual mice that yield half-maximal binding to the immunizing antibody immobilized on the plate. Thus, sera from MoV_{NP}-Moγ2b^b-immunized mice were tested on an MoV_{NP}-Moγ2b^b coat, etc. Lightly crosshatched bars give titres for the primary response; stronger crosshatching indicating the increase in the secondary response. A bar indicates the titres obtained from the preimmune sera. Where there was no significant difference between the primary and secondary responses, only the secondary is depicted.

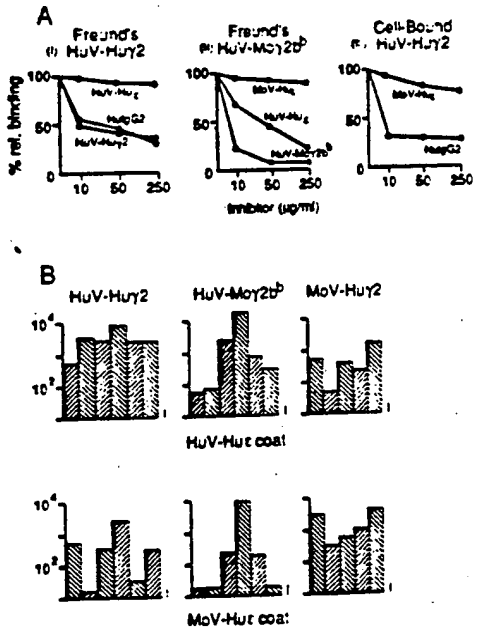

FIGURE 3. Specificity of the antiantibodies. (*A*) Binding inhibition assays. The bindings of antiantibodies in a serum dilution from individual mice hyperimmunized with (*I*) HuV_{NP}-Huγ2 in Freund's, (*II*) HuV_{NP}-Moγ2b^b in Freund's, or (*III*) cell-bound HuV_{NP}-Huγ2 were tested on a coat of the immunizing antibody in the presence of various concentrations of competitor. The result is given as the percentage binding relative to that obtained in the absence of inhibitor. Inhibition assays shown are for individual mice but are representative of the three in each group tested. (*B*) Direct binding of sera from mice hyperimmunized with HuV_{NP}-Huγ2, HuV_{NP}-Moγ2b^b, or MoV_{NP}-Huγ2 antibody in Freund's to a MoV_{NP}-Hue or HuV_{NP}-Hue coat; binding could not be inhibited with a human IgE myeloma protein. Bars for individual mice titred on MoV_{NP}-Hue are aligned with bars for the same mice titred on HuV_{NP}-Hue.

markers, mice were challenged with syngeneic spleen cells to which antibody had been bound. While the responses were considerably weaker than to the antibodies administered in CFA, the cell-bound xenogeneic and chimeric antibodies nevertheless elicited a clear response with the major part of the response to HuV_{NP}-Huγ2 being directed against the C region (Figs. 3 *A* and 2 *B*). Within the variation from individual animals, there was no clear difference in the immunogenicity of the xenogeneic and chimeric antibodies. The contrast between these results and those obtained using Freund's might be accounted for by the fact that mouse IgG2b, but not human IgG2, binds to some mouse Fc receptors (6).

Although administration of a syngeneic antibody need not elicit an antiantibody

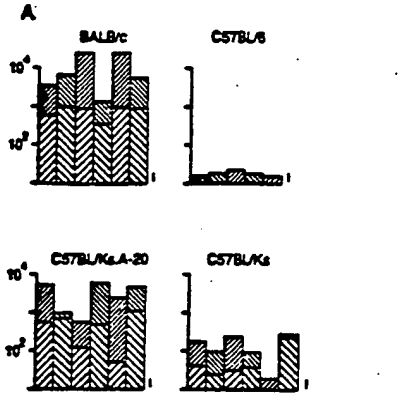

FIGURE 4. Allogeneic responses. (*A*) The response of C57BL/6, BALB/c, C57BL/Ks, and C57BL/Ks.A-20 mice to immunization intraperitoneally with MoV_{NP}-Moγ2b^b antibody in Freund's. (*B*) Binding inhibition assay of the antiantibody response in one of the MoV_{NP}-Moγ2b^b-immunized BALB/c mice. Of three other mice tested, two gave curves similar to those presented, whereas one showed a greater degree of inhibition by MoV_{NP}-γ2b^b than by MoV_{NP}-Hue.

response, polymorphism within the human population may lead to responses even to wholly human antibodies. To compare the magnitude of such an allotypic response with the response mounted against foreign V region frameworks, MoV_{NP}-Moγ2b^b was injected into both C57BL/6 (the strain from which the antibody originates) and BALB/c mice. Unlike C57BL/6, the BALB/c mice made a strong response against MoV_{NP}-Moγ2b^b, recognizing both V and C domains (Fig. 4 *A*). Although immune response genes could well play a role (7), the difference in the response obtained with the G57BL/6, BALB/c, and F_1 mice is likely to be due to the difference in Igh haplotypes. This was confirmed by comparing the responses of C57BL/Ks (H2^d, Igh^b) with C57BL/Ks.A-20 (H2^d, Igh^a) mice (Fig. 4 *B*).

Thus, an antibody with both foreign C_H domains and foreign V_H frameworks was strongly immunogenic, eliciting a response that was largely directed against the C region but with a substantial component against the V. In a chimeric derivative (in which only the V region frameworks are foreign), the anti-C response was abolished but the response to the V remained and was unattenuated. While all foreign framework sequences may not prove equally immunogenic, the results indicate that, short of administering an autologous antibody, therapeutic applications should make use of antibodies in which care has been taken to reduce the V region immunogenicity. However, the immunogenicity of antibodies in which the hypervariable regions are the sole foreign determinants is an unknown quantity and is an important focus for further research. Extrapolating to therapy in man, the results caution that, even with wholly human antibodies, problems may be encountered with allogeneic responses directed against both the V and the C. Ultimately, it may prove advisable not just to use humanized antibodies, but to use antibodies whose allotype is matched to that of the patient.

Summary

Mice were immunized with model xenogeneic (both the V_H frameworks and the C_H domains of human origin), chimeric (just V_H frameworks human), or self antibodies, and the antiantibody responses were dissected. Only the self antibody did not elicit a response. A strong response was elicited by the most xenogeneic antibody with ~90% against the C and ~10% against the V. The anti-V response was not

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attenuated in the chimeric antibody, demonstrating that foreign V_H frameworks can be sufficient to lead to a strong antiantibody response. The magnitude of this xenogeneic anti-V_H response was similar to that of the allotypic response elicited by immunizing mice of the Igh^a allotype with an Igh^b antibody. Thus, although chimerization can diminish antiantibody responses, attention should be paid both to V region immunogenicity and to polymorphism.

We thank Phil Wright, Catherine Teale, and Gareth Williams for their help.

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Immunotherapy and other novel therapies

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Although no true breakthroughs occurred, publications during the 12-month period of this review added substantial definition to certain novel immunotherapies potentially applicable to the treatment of rheumatoid arthritis. Overall, this period witnessed maturation in the field of biologic interventions. Clinical trials provided further data needed to assess the efficacy of high-dose intravenous γ-globulin therapy in patients with systemic juvenile rheumatoid arthritis, and extended uncontrolled experience with interferon-γ in adult rheumatoid arthritis was obtained. An intriguing immunostimulant and antiviral drug, isoprinosine (inosine pranobex), failed in a scientifically rigorous trial in rheumatoid arthritis. Provocative insights into totally new approaches surfaced in additional reports from a variety of immunologic areas. Although seemingly distal to rheumatoid arthritis, these papers are cited because their further development or adaptations could reach a stage where clinical trials in rheumatoid arthritis are warranted.

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Intravenous gammaglobulin therapy

Diseases for which high-dose intravenous γ-globulin therapy might be effective continue to proliferate. In a pilot study, seven of eight patients with treatment-resistant active juvenile rheumatoid arthritis, including systemic features, responded to a regimen similar to that in standard use for idiopathic thrombocytopenic purpura and other autoimmune disorders [1••]. Although open in design, most of the clinical outcome variables measured changed substantially, lending credibility to a conclusion that the intervention did indeed modify the disease. Improvement in fairly objective parameters, including the erythrocyte sedimentation rate and hemoglobin and albumin levels, were noted as well. The major problems with intravenous γ-globulin therapy seem to be monetary and logistic. Although the mechanism(s) responsible for the salutary effect remains elusive, additional evaluations of intravenous γ-globulin therapy in juvenile rheumatoid arthritis clearly appear to be indicated.

Pulse methylprednisolone or nitrogen mustard therapy

Although practiced not infrequently by certain experienced clinical rheumatologists, there is little literature on pulse treatment with methylprednisolone or nitrogen mustard. Thus, the review from the Cleveland Clinic Foundation is of importance [2•]. Both methodologic details and clinical and immunologic sequelae of high-dose intravenous pulse steroid and chemotherapy are detailed in a nonjudgmental fashion in this article. Based on their experience and the data displayed in the independent reports cited, the efficacy to toxicity ratio of nitrogen mustard appears to be acceptable for patients whose rheumatoid arthritis is refractory to more conventional approaches. The ready availability of mustard to rheumatologists would seem to justify further attention to this relatively neglected area of therapeutics, if a truly scientific format is followed.

Recombinant interferon-γ

Subcutaneous administration of recombinant interferon-γ as a potential treatment for rheumatoid arthritis has exhibited a discordant record in clinical studies to date. All uncontrolled trials have been short term, and although improvement has been described, benefit occurred in only two of the four placebo-controlled studies. Thus, the extended, open-label, 2-year follow-up of patients who participated in a prospective double-blind US multicenter trial is of interest [3•]. At 1- and 2-year follow-ups, 57% and 38% of the patients, respectively, remained on the agent. Improvement, compared to study entry, was evident in perhaps one third of the patients at 1 year and in 15% to 20% at 2 years. Systemic side effects (fever, chills, headache, nausea, diarrhea, and local reactions at the injection site), so troublesome in the short-term studies, seemed to abate, with only fever being a significant problem.

Unfortunately, plaudits stop here. A number of major weaknesses make the study extremely difficult to interpret. Concurrent therapy with a gamut of drugs was permitted; the majority of patients were receiving nonsteroidal anti-inflammatory drugs, prednisone, and one of several so-called remittive regimens (gold, penicillamine, hydroxychloroquine, and even gold plus hydroxychloroquine). Even more confounding is the variable decrease in dose and frequency of interferon-γ that these patients received. It is likely that less exposure to the agent explains the better "tolerability" to the therapy. It is much more difficult to identify the drugs that were responsible for the improvement in this cohort.

Isoprinosine fails

The past decade has seen a series of putative immunoenhancer and antiviral agents surface as dualistic approaches for the curtailment of rheumatoid arthritis. Levamisole and amiprilose were two of the more widely touted drugs of this genre. During this period, another candidate known by a variety of names, including isoprinosine, inosine pranobex, inosiplex, and even methisoprinol slowly matured along this pathway. Lymphocyte stimulatory and antiviral properties were defined, but unfortunately most of the work was outside of the boundaries of peer-reviewed journals. Purportedly beneficial effects in rheumatoid arthritis appeared a number of years ago in an open study, prompting a careful, large-scale scrutiny within a double-blind, placebo-controlled format at the prestigious Center for Rheumatic Disease in Glasgow [4•]. Unfortunately, no significant improvement in any disease variable was noted in this 24-week trial. Equally intriguing was the fact that no evidence of spontaneous improvement in the disease occurred in the placebo limb, unlike the response to placebo in several recent trials in the United States. Are the Scots less suggestible, or has the so-called "placebo effect" been exaggerated recently?

Misoprostol: a gastroenterologic bequeathment to rheumatologists?

Pioneering, but often overlooked, work performed two decades ago elucidated striking anti-inflammatory properties for prostaglandins at pharmacologic doses in the adjuvant model of arthritis. In a variety of contexts, additional tantalizing properties for prostaglandin analogues have surfaced and engendered interest in their potential role in the treatment of immunologically mediated inflammatory arthritis [5•]. The most extensively studied candidate is misoprostol, the prostaglandin E_1 analogue now widely used to thwart nonsteroidal anti-inflammatory drug–induced gastropathic bleeding. An additional impetus to this theory has been several disclaimers of the "Vane hypothesis," *ie*, that prostaglandins are the major inciters of inflammation and their attenuation explains the efficacy of nonsteroidal anti-inflammatory drugs in inflammatory arthritis. Now that the precise mechanism of action of nonsteroidal anti-inflammatory drugs seems to have fallen into a "black hole," the once-antithetical possibility that prostaglandins might be clinically useful anti-inflammatory drugs is being considered. How this might be accomplished is a mystery. Could high ambient levels of prostaglandins inhibit macrophage or polymorphonuclear leukocyte functions, such as chemotaxis? Alternatively, could this feat be accomplished by immunoregulatory actions? *In vitro*, misoprostol has been reported to suppress lymphocyte proliferative responses. *In vivo*, misoprostol administration promoted the ability of humans to retain renal allografts. Further exploration into the possibility that prostaglandin E_1 analogues alter T-cell processes could bring about a new therapeutic dimension for patients with rheumatoid arthritis far beyond their action on the gastric mucosa.

Biologic inhibitors

The new frontier of intervention into autoimmune disease by specific, targeted biologic products (the plentitude, purity, and specificity of which are tightly insured by production via recombinant technology) continues to emerge. Potential applications, including osteoarthritis as well as rheumatoid arthritis, were recently reviewed by Hess [6•]. In terms of actual development, recent media attention has been devoted largely to a recombinant protein able to specifically inhibit the alleged central culprit in rheumatoid arthritis: interleukin-1. Paralleling this effort has been the purification of a natural inhibitor of another cytokine, tumor necrosis factor [7•]. Although the relevance of tumor necrosis factor and the biologic outcome of its banishment by a monospecific inhibitor remain in doubt, the isolation of interleukin inhibitors strengthens the probability that interleukin-mediated processes all involve precise cell surface receptors, and abrogation of the activity can be achieved by intervening with either the factor or its surface receptor.

The recent elucidation of a contra-interleukin-2 cytokine in the mouse [8•] further illustrates this theme. Based on the importance of T cells and probably interleukin-2 in the pathogenesis of the collagen model of rheumatoid arthritis, clones of T cell from collagen-immunized mice were probed and some were found to secrete a factor that specifically antagonized interleukin-2-mediated pathways *in vitro*. Clinical assessment in the model revealed that inflammation could be reduced by injecting this protein. Although the exact biochemical nature of this adversarial product is uncertain, extrapolation of this approach to the future treatment of rheumatoid arthritis can be envisioned.

Another report [9•] interjected caution into these approaches. While specific biologic antagonists for the immune system are being designed, often in "high-tech" fashion, naturally occurring counterparts are being elucidated as well. The existence of these naturally occurring contra molecules could argue that treatment with "designer molecules" would be superfluous and therefore ineffective. The recognition that autoantibodies of the IgG isotype capable of neutralizing the activity of interleukin-1 can be found in the sera of some patients with rheumatoid arthritis [9•] would argue that in certain stages of the disease, the body has already at least partially checkmated the process. Thus, additional therapeutic intervention would be biologically effete. Unidimensional attacks on aberrant immune pathways might have a limited effect on the underlying disease process.

Another example of a highly targeted approach is the use of a novel recombinant fusion protein with impressive specificity for the high-affinity interleukin-2 receptor. DAB_{486} interleukin-2 is a conjugate consisting of a portion of interleukin-2 joined to a diphtheria toxin fragment. This amalgam enables the hybrid species to interact as a ligand with the cell surface receptor for interleukin-2 and then deliver a lethal hit. Activated T cells express the interleukin-2 receptor, causing the molecule to be therapeutically appealing. Recent evaluation in adjuvant arthritis indicated that use of the protein was capable of suppressing both the induction and established stage of this model [10•]. By inference, these data strongly imply that interleukin-2-positive cells orchestrate a major portion of the pathogenesis of this disease. A theoretic concern with the use of this diphtheria conjugate to treat autoimmune disease in humans is their ubiquitous prior exposure to diphtheria vaccines, *ie*, that circulating antibodies to diphtheria might latch on and capture the immunologic missile before it could encounter the target cells. To address this potential liability, rats were preimmunized to diphtheria before the attempted induction of adjuvant arthritis. Even in these rats, where appreciable titers of diphtheria antibodies were found, the interleukin-2 receptor immunotoxin was effective. Inceptual feasibility work in rheumatoid arthritis was begun, and additional information regarding prospects for this approach should soon be available.

Hypothetical vaccination strategies also reside within the realm of targeted biologic therapies. Candidate autoantigens have been identified, including type II collagen and heat shock protein in rheumatoid arthritis. Schemes by which antigen-specific immunosuppression can be achieved have also been established, such as intravenous T-line cell inoculation. Earlier work showed that intravenous injection of antigen-coupled mononuclear cells or erythrocytes could abort an immune response to the antigen. Type II collagen coupled cells, injected intravenously, were used to attenuate the onset of adjuvant arthritis, demonstrating that intrinsically similar pathways operate in both the collagen and adjuvant models. Practicality, however, was lacking in this approach. More recently, investigators have unearthed another approach harkening back to older literature that cited peroral allergen administration as capable of expurgating atopic responses. For example, ingestion of poison ivy was claimed to be a way to alleviate contact sensitivity to this stimulus. The basic tenant is that gut lymphoid tissue is preferentially primed towards suppression of immune responses. Recent work has shown that experimental allergic encephalomyelitis and collagen arthritis can be prevented by peroral administration of myelin basic protein and type II collagen, respectively. Innovative experiments by Zhang *et al.* [11••] have shown that type II collagen ingestion can also downregulate adjuvant arthritis, duplicating the earlier results with collagen-coupled cells. Additional experiments revealed that immunosuppression was antigen specific and capable of being adoptively transferred with cells that were probably T suppressor in nature [11••]. The safety and simplicity of this vaccination protocol have been sufficiently compelling to warrant study in humans, seeking to ascertain whether curtailment of autoimmune disease in humans can be achieved by this approach. Major unanswered questions at present include 1) whether the human immune system recapitulates that of the mouse and therefore can be downregulated by oral antigen delivery, and 2) whether an established immune process, as is operative in autoimmune disease, can be quiesced by oral antigen intake.

Ideas for potential biologic approaches do not come solely from molecular biology laboratories. Harris and Sledge [12•] recently pinpointed in print the intriguing phenomenon that artificial hip replacement in patients with rheumatoid arthritis consistently results in sustained abatement of inflammation in the joint, whereas a similar outcome is not evident with knee replacement. In their opinion, the major differences in the two procedures relate to the complete absence of residual cartilage after a hip replacement, whereas patellar cartilage remains in a total knee joint replacement. Does the lack of cartilage explain the alleviation of synovitis after hip replacement? This experience seems sufficiently provocative to warrant further scrutiny into the precise nature of the provocative material in cartilage. Is it collagen? Is it proteoglycan? Is it possibly even a sequestered infective agent or by-product?

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Possibly an important clue furnished by surgeons who keep their eyes open and minds engaged.

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